

Investigation of Alternative Methods for the Synthesis of 1,2,3-Triazole Analogues of Combretastatin A-1 and A-4

Linda Dohrn



SECTION OF MEDICINAL CHEMISTRY
DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
SCHOOL OF PHARMACY

UNIVERSITY OF OSLO

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Abstract

1,2,3-Triazole analogues of Combretastatin A-4 have interesting tubulin inhibition and cytotoxic effects against several cancer cell lines.

These triazoles can be attained by either the thermal Huisgen cycloaddition reaction and by a magnesium catalyzed method. Both methods have disadvantages; thermal cycloaddition yields two regioisomers and the magnesium catalyzed reaction is intolerant with substituents, such as the nitro-group.

In this thesis alternative methods for the synthesis of 1,2,3-triazole analogues of Combretastatin A-1 and A-4 were investigated. In the presence of *tetra*-ethyl ammonium hydroxide the desired 1,5-disubstituted 1,2,3-triazole analogues were attained.

Abbreviations:

CuAAC: copper catalyzed azide-alkyne cycloaddition

RuAAC: Ruthenium catalyzed azide-alkyne cycloaddition

Ligand d: (1*S*,2*S*)-*N*¹,*N*²-dimethylcyclohexane-1,2-diamine

Ggt: *guttae* = drop (latin; drop of liquid)

1. Introduction

The development of angiovascular disruptive agents is a promising approach to cancer treatment. Tumors will fail to develop past a size of 1-2 mm³ without sustained angiogenesis. Vascular disruptive agents like combretastatins have shown to selectively disrupt existing blood vessels and cause vessel collapse leading to tumor necrosis.

Combretastatins belong to a class of natural products which target microtubuli in cells and show potent cytotoxic activity. Combretastatins exerts a destabilizing effect on the tubulin heterodimer which therefore fail to polymerize to microtubuli. Microtubuli is an important cellular component responsible for chromosomal separation during mitosis, cellular shape and morphology, and intracellular transportation of vesicles and organelles. Cells unable to perform mitosis enter mitotic arrest and perform controlled cell death. The combretastatins have shown high selectivity towards vascular endothelium in tumors, causing rapid vascular collapse and tumor necrosis.

The development of angiovascular disruptive agents is a promising approach to cancer treatment. Tumors will fail to develop past a size of 1-2 mm³ without sustained angiogenesis. The interest in combretastatins is therefore as vascular disruptive agents in chemotherapy. The combretastatin stilbene is a simple structure that is amendable for isosteric substitution and *structure-activity relationship* studies.

It is part of this aim to develop new tubulin inhibitors based on the combretastatin A-series amendable for biological testing and SAR-elucidation.

This thesis approaches the synthesis of new combretastatin derivatives through triazole formation by azide-alkyne cycloadditions (AAC). The synthesis of 1,4-disubstituted triazole analogues were performed with the regiospecific copper-catalyzed AAC, which is a simple and reliable method to procure 1,4-diaryl-1,2,3-triazoles. A new regiospecific approach towards the synthesis of 1,5-diaryl triazole analogues was also tested.

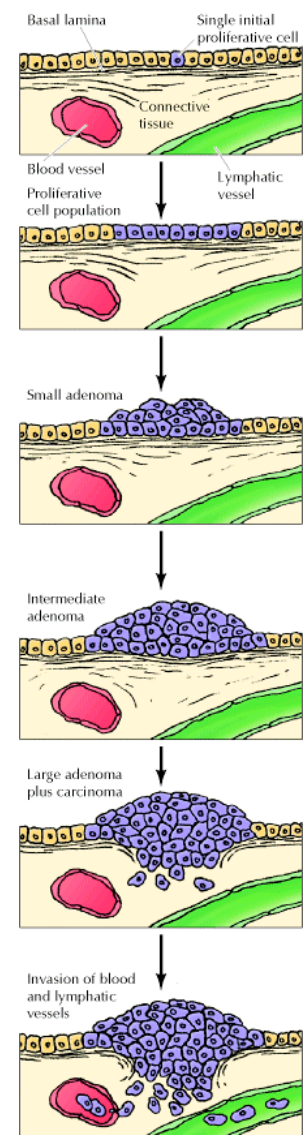
Biological testing and SAR-elucidation was not performed as time did not allow for this.

1.1. Cancer

Tumor development and certain retinal degenerative disorders display abnormal angiogenesis which play a pivotal role in the progression of these diseases. There are more than a hundred distinct types of cancer, which vary greatly in behaviour and response to treatment. Cancer pathology differs between benign and malignant tumors. A benign tumor has a distinct perimeter, and remains confined to its original location without invading surrounding tissue or spreading to other sites in the body. Benign tumors are not considered as cancer, and therapy is usually constricted to surgical removal if tumor size exerts pressure to other organs, causes pain or suppresses blood supply. A malignant tumor has a diffuse perimeter, is capable of invading surrounding tissue, and penetrates the circulatory or lymphatic system to spread throughout the body. Such tumors are referred to as cancers, and are difficult to treat when metastasis has occurred. Where surgery can eradicate solid tumors of a certain size and location, it requires removal of surrounding tissue to exclude survival of invasive cancer cells in the periphery. It has limited effect once metastasis has occurred, if tumor-location contraindicates removal or the cancer is systemic. Radiotherapy is mostly dependant on the generation of hydrogen-reactive species formed from oxygen, such as hydrogen peroxide, and hypoxic cells are less affected than well-oxygenated ones. Radiotherapy is not suited for systemic cancers such as leukaemia. There are several chemotherapeutical agents that rely solely on vascularisation for delivery and therefore have limited effect on badly vascularized cells within a tumor. Circumventing this dilemma is direct drug-injection into the tumor that again requires a tumor of certain size and location. Chemotherapies based on metabolic rate or active proliferation will not target cells in senescence, a state often triggered due to hypoxia and metabolic starvation.

Tumour initiation begins as a genetic alteration in a progenitor cell that leads to abnormal proliferation and the outgrowth of a population of clonally derived tumor cells. Tumor progression continues as these clonally derived cells continue to proliferate, and a neoplasm forms. Some of the mutations deriving from the proliferative rampage will confer a selective advantage to that cell, and the descendants of that cell will become dominant within the tumor population as a cause of this. Such an advantage can be more rapid growth, or decreased sensitivity to apoptotic signals, and is termed clonal selection. Thus clonal selection continues throughout tumor development, turning them more rapid- growing, increasingly malignant, and may even cause therapy-resistant populations.

Delivery of nutrients is initially performed by existing vasculature that may manage to sustain the tumors metabolic demands up to the size of a few millimetres. Expansion beyond this point requires formation of new blood vessels, and the tumor becomes increasingly famished and hypoxic without neovascularisation. The tumor will fail to develop further, and enters a dormant state unless it surpasses the angiogenic switch. The increased hypoxia and nutritional deprivation activates angiogenesis through a series of growth signals. As new blood vessels form the capillaries may consist partly of endothelial cells and cancer cells, forming a mosaic wall. This, and penetration of the pre-existing circulator system, allows for cancer cells to spread and metastase. (Picture [1])



1.2. Tumor angiogenesis

Tumors develop a high metabolic demand which increase dramatically past a size of 1-2 mm³. Past this size the existing vasculature fails to deliver sufficiently the necessary nutrients and oxygen, and the tumor becomes hypoxic and starved. The tumor mass suffers greatly from cell death as it becomes rapidly more famished and metabolic waste accumulates. This state triggers several factors that result in neoangiogenesis, and is termed the “angiogenic switch”. Hypoxia is among the signals inducing cell-secretion of growth factors and various other stimuli resulting in neoangiogenesis. Continuous vascularisation during tumor expansion represents a promising target for anticancer drug development. [2]

1.3. Organization and vascularisation of tumor vessels

The rapid growth and physiological conditions during cancer angiogenesis result in morphological traits differing greatly from normal vasculature. Under normal conditions the vessels organize in an orderly manner with adjacent cells lying closely together providing an even and smooth vasculature wall. Nutrients and oxygen pass evenly, and distribution is well developed. Vessel organization in tumors is aberrant and generates hypoxic conditions due to the irregular, sluggish and intermittent blood flow, with an uneven diameter and wide junctions at some regions and stacked layers of endothelial cells at others. Common features are dilated and elongated shapes, with dead ends and leaky sprouts. As the endothelial cells frequently undergo apoptosis they expose cancer cells to the lumen. Incorporation of cancer cells into the vascular wall gives a mosaic and tortuous appearance, expressing both endothelial and cancer cells at varying ratios. [2, 3]

Tumors become vascularized by either de novo formation of new vessels or acquisition and expansion of pre-existing vessels. The mechanisms involved are defined as “sprouting” angiogenesis, intussusceptive angiogenesis and by angioblast recruitment. Sprouting angiogenesis is stimulated when angiogenic growth factors emitted by the tumor activate receptors on pre-existing vasculature. The activated endothelial cells release metalloproteases that degrade the extracellular matrix in order for migration of cells towards the angiogenic stimulus, as well as proliferate as a response to tumor secreted VEGF.[3] The endothelial cells migrate in tandem, and form loops to create the lumen. With the aid of integrins the cells manoeuvre and adhere to adjacent cells. Sprouting angiogenesis can develop at a rate of several millimetres a day, and creates completely new blood vessels. Intussusceptive angiogenesis is also known as splitting angiogenesis, where the capillary wall extends into the lumen to split the existing vessel in two.

Under these conditions the tumor consists of both well vascularised tumor cells and hypoxic, nutrient deprived cells that ultimately determine the response and effect of cancer therapies. The physiological conditions described in tumor treatment often necessitate a multi-dose combinatorial regime to avoid the possibility of tumor recovery. With the pivotal role tumor vascularisation play in both progression and therapy resistance in cancers it has received massive attention as a key target in future treatments.

1.4. Antivascular agents

The role and mechanism behind tumor initiated angiogenesis have launched massive research to find highly specific cancer treatments. [2] There are two main classes to consider, the *angiogenesis inhibitors* (AIs) and the *vascular disrupting agents* (VDAs). The two categories differ in that AIs interfere with the formation of tumor vasculature, thus halting tumor growth and metastatic spread, while VDAs cause rapid shutdown of established tumor vasculature, leading to ischemia, cell death and thus tumor mass destruction. Strategies included in the AI approach includes agents that interfere with key signalling pathways in intracellular communication and angiogenic stimuli, such as excretion and diffusion of VEGFs, the use of antibodies to inhibit or inactivate factors after their release and receptor-inactivating substances. The category also embraces agents that interfere with endothelial cell proliferation, migration and tissue-invasion. VDAs can be categorized into substances that deliver toxins or effector molecules to tumor endothelial cells (like antibodies and peptides), and small molecules that induce selective vascular shutdown by exploiting the physiological differences between normal and tumor-associated vascular endothelium.[2]

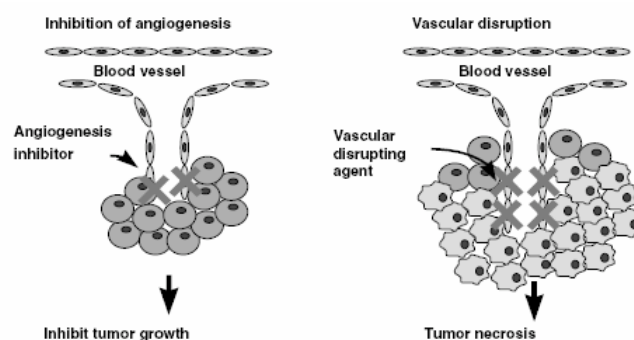


Figure 1 Differences in the mechanism of action and antitumor effects between AIs and VDAs[4]

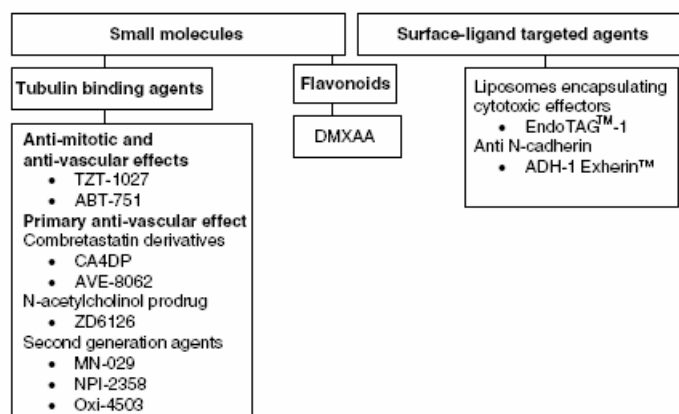


Figure 2 Vascular disrupting agents in clinical development per 2007.[4]

1.5. The cell cycle and regulation, senescence and apoptosis

Central to the understanding of carcinogenesis are the cellular mechanisms concerning the cell cycle, senescence and apoptosis. The complex regulatory machinery involved in these phases has proven important key features in the development of cancer, and offers several potential targets for chemotherapy. A short introduction to the molecular bases of cancer is therefore offered, with an overview of the characteristic of cancer cells and tumours and how it differs from normal cells and tissues. A more thorough presentation of angiogenesis is linked to the development of vascular-targeting agents such as the combretastatins. Key aspects to cancer treatment are also presented, along with important reasons for treatment failure.

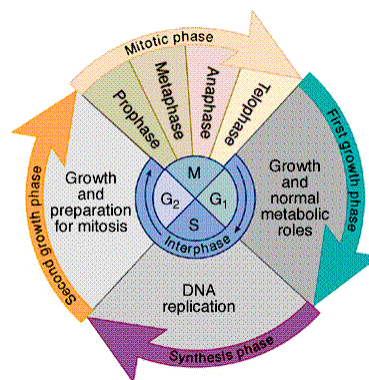


Figure 3 Overview of the cell cycle [5]

The cell cycle represents a series of events taking place during cell replication. It is divided into four major phases termed sequentially G₁, S, G₂ and M. In a simplified manner the process of mitosis and cytokinesis occur during M phase and DNA replication performs during S phase. Cells that no longer proliferate exit the cycle and enter a quiescent state called G₀ or senescence. The phases G₁ and G₂ (G stands for Gap) are regulated by factors that induce or suppress cell cycle progression, and are stages in which the cell prepares for entry into the next phase. In G₁ the biosynthesis of various enzymes and proteins necessary for DNA replication increase, and during G₂ synthesis of proteins such as microtubule scaffolding and other functions necessary for mitosis occur. During M phase the separation of chromosomes between daughter cells is orchestrated by microtubuli and centromeres, and is further divided into four phases, pro-, meta- ana and thelophase, followed by cytokinesis. [1]

The chromosomes condense and centrosomes move to opposite sides of the nucleus during pro-phase, and formation of the mitotic spindle is initiated. The nuclear envelope degrades and allows spindle microtubuli to attach to the kinetochores of chromosomes. The

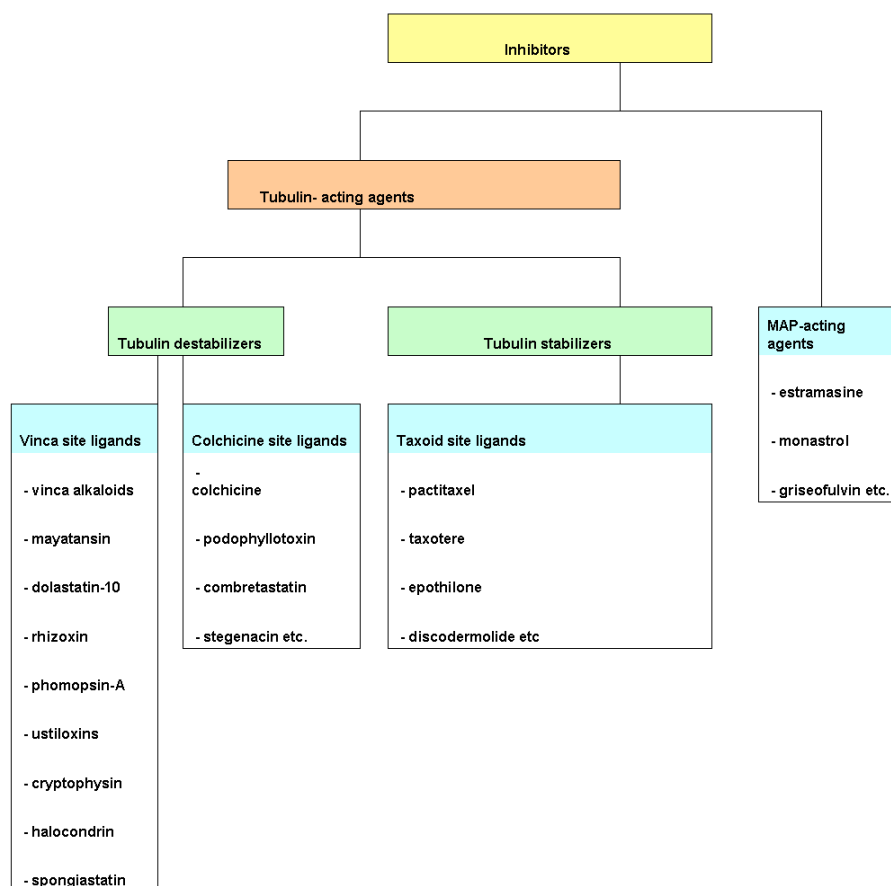
chromosomes shuffle back and forth between the centrosomes and the center of the cell, an event sometimes referred to as prometaphase. At the stage where the chromosomes eventually align at the center of the spindle it reaches metaphase. Next, in anaphase, the sister chromatids separate and move to opposite poles of the spindle. Telophase states the end of mitosis as the nuclear envelopes forms and the chromosomes decondense. Cytokinesis takes place parallel to telophase, and divides the cellular contents by pinching of the cellular membrane to form, and finally separate, the two daughter cells. They each contain one chromatide, and are able to enter G_1 or G_0 according to proper stimuli. [1]

1.6. Microtubuli, tubulin and tubulin binding sites

Microtubules are involved in several important processes beside cellular division, such as intracellular transport of vesicles and organelles, and as part of the cytoskeleton. Microtubuli are stiff and hollow cylindrical polymers where the walls comprise of 13 protofilaments. Protofilaments are made of tubulin, a heterodimer of α - and β -tubulin that arrange the N-terminal in α - tubulin against the C-terminal in β -tubulin. The microtubuli thus exerts polarity with a plus and minus end. Between the protofilaments the C-terminal of α -tubulin interact with the N-terminal of β -tubulin thus further stabilizing the lattice within the structure. The binding energy associated with these protein-protein interactions are strong enough to ensure that the dynamic growth and loss of tubulin almost entirely occur at the microtubule ends. The growth and polymerization of tubulin to microtubule requires two bound energy rich guanosine triphosphate (GTP) to the heterodimer. The α - unit irreversibly bind to the GTP-molecule, while the β -unit may freely exchange between GTP and the hydrolyzed counterpart guanosine diphosphate (GDP). The growth is dependant on a more rapid assembly of GTP-bound tubulin relative to the hydrolysis of GTP, which causes a conformational change in the subunit and weakens the protein-protein bonds. The subsequent curving of the protofilament causes depolymerisation of the microtubule and shrinkage. There are several specific isotypes of the α , β -heterodimer which vary between different types of cells. The isotypes differ in degree of stability, and may even contribute to higher tolerance to tubulin-acting agents. [4, 5]

The important role of microtubuli in cells makes them promising drug targets, and a large number of naturally occurring compounds that disrupt the dynamic stability have been discovered. Tubulin inhibitors lead to mitotic arrest and apoptosis when the cell fails at proper formation of the spindle and chromatide separation. The effects on cellular structure can be fatal on endothelial cells, and further described under effects of vascular disruptive

agents. Three distinct binding sites for tubulin-acting agents have been characterized, and include the colchicine and vinca alkaloid domain located on the monomere subunits of tubulin, and the taxoid binding region found on polymerized microtubuli. Agents that bind to the vinca domain include the natural products vinblastine and vinkristine, with derivatives, which destabilize the formation of microtubuli. The colchicine binding site is named after colchicine, which binds irreversibly to the site in a destabilizing manner. The domain is also targeted by combretastatins that bind reversibly to the site. Combretastatins also yield a better toxicity-profile compared to colchicine-analogues, as cytotoxic effects on normal cells are limited to duration of exposure. Dissociation of microtubuli is seen close to the *maximum tolerated dose* (MTB) for colchicine and several of the other natural spindle poisons, while as the combretastatins exerts effect at much lower dosages than their established MTD. Among ligands for the taxoid binding site are paclitaxel, taxotere, discodermolide and epothilones, which stabilize the microtubuli to continuously expand its positive end until apoptosis occur. A table over some tubulin-acting agents is depicted in the figure.



1.7. Tubulin inhibitors

The naturally occurring tubulin inhibitors often display highly complex structures with exceedingly difficult synthetic routes in the laboratory. The compounds show low bioavailability due to their lipophilic nature, and often exert tubulin inhibition at doses close to their MTD as noted. Combretastatin is a simple stilbene in comparison, which may easily be diversified to evaluate its tubulin-binding capacity.

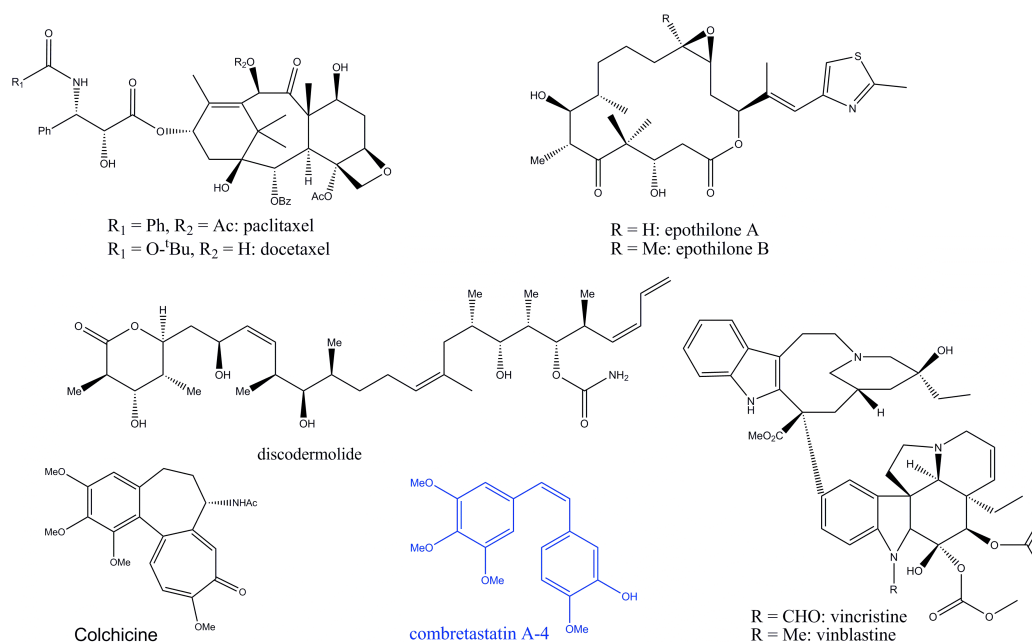


Figure 4 Selected tubulin inhibitors and their structures

1.8. Combretastatins

Combretastatins belong to a class of natural products isolated from the South African tree *Combretum caffrum*. This class of compounds has been identified as competitive inhibitors of the colchicine binding site on β -tubulin, one of several distinct binding regions on this protein defined by its affinity for colchicine.

The family of combretastatins, not to be confused with the cholesterol-lowering agents also termed statins, exerts their effect predominantly on tumor associated vasculature where they target tubuli and destabilize it. Tubulin acts as the fundamental element in microtubuli, a cellular construction responsible for several important functions within the cells architecture

and intercellular transportation, and plays a pivotal role in the proliferation process. Thus, tubulin acting agents target predominantly the mitotic spindle during proliferation, and the structural shape of the cell. The result is a cell incapable of separating the chromosomes nor able to perform cytokinesis. An even more profound effect is the loss of cellular shape due to the altered cytoskeleton. Recent reports suggest this is due to reorganization of the actin matrix leading to formation of large “blebbing” cells lacking firm attachment to other endothelial cells, as well as detachment to the basal lamina.[6] Complete detachment of cells will ultimately lead to vessel collapse, and the increased gaps between such enlarged cells will increase the vascular permeability of larger proteins and molecules. This enables chemotherapeutics to transfer more selective to cancer cells as the veins are more permeable to larger chemical structures that would not pass normal vasculature endothelium. Loss of vascular integrity leads to reduced blood pressure and blood flow, causing red blood cells to stack up in coin-like formations further blocking the blood vessels. The tumor suffers greatly from ischemia and necrosis when the vasculature undergoes these processes. With this angle of approach the cells at the perimeter supported by normal vasculature is less effected than the inner tumor mass, leaving a rim of cancer cells viable for tumor recovery. Such well-vascularized cells are more susceptible for traditional chemotherapy, and as the two therapies are complementary the VDAs are now administered following either chemo- or radiotherapy in clinical development programs without increasing the toxicity of the chemotherapeutical agents. The combretastatins show clinical effect at dosages far below the maximum tolerated dosage (MTD) with high toleration among patients.

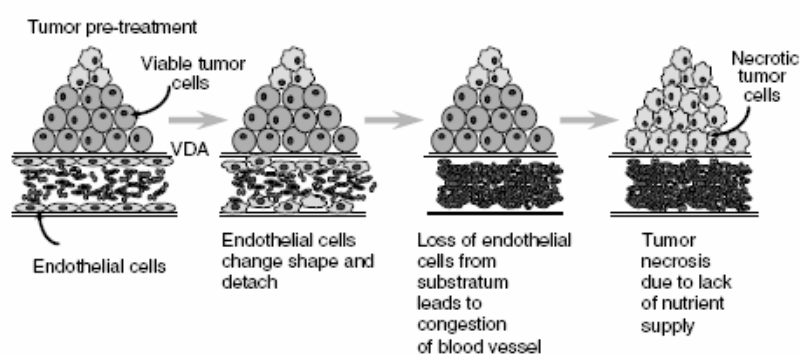


Figure 5 Mechanism of action of vascular disrupting agents (VDAs).[4]

The combretastatins consists of several structurally different natural products falling under the categories A, B, C and D-series according to their structural characteristics. The A- series consist of the *cis*-stilbenes, the B-series of diaryl-ethylenes, C-series of quinones and the D-series comprise the macrocyclic lactones. [7]

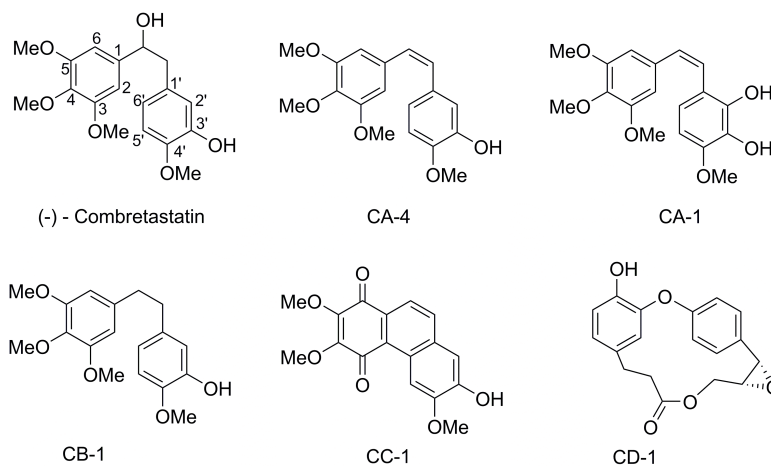


Figure 6 Selected analogues from the combretastatin series comprising of A, B, C and D-series.

The most potent compound in this family has shown to be the monophenolic combretastatin A-4, which exhibits a potent cytotoxicity towards a broad array of human cancer lines, as well as not being a substrate for the multidrug resistance (MDR) pump. The promising results for CA-4 have launched extensive studies to reveal its pharmacophore, which have also proven beneficial for the development of combretastatins of the A-1 series. Combretastatin A-1 is the catechol counterpart to CA-4, which also show significant inhibition of cancer cell growth and antimitotic activity. According to structure-activity-relationship (SAR) studies the 3,4,5-trimethoxy substituent's on the A ring are essential as well as the *cis*-configuration. The B-ring show more tolerance towards structural modifications as the 3-hydroxy group(s) is optional, but the *para*-positioned methoxy group is required for potency. [8]

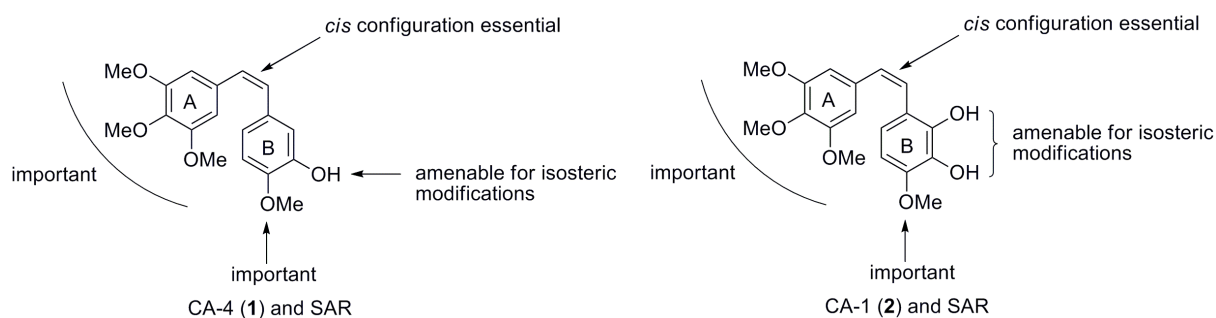


Figure 7 Known SAR of CA-4 and CA-1 analogues

Although the naturally occurring combretastatins exhibit potent biological activity *in vitro*, they suffer from poor bioavailability *in vivo* due to their lipophilic nature and low aqueous solubility. [9, 10] Combretastatins can also form phenantrenes that show reduced cytotoxicity. The catechol moiety in CA-1 is sensitive to oxidation to 1,2-benzoquinones which also show reduced cytotoxicity.

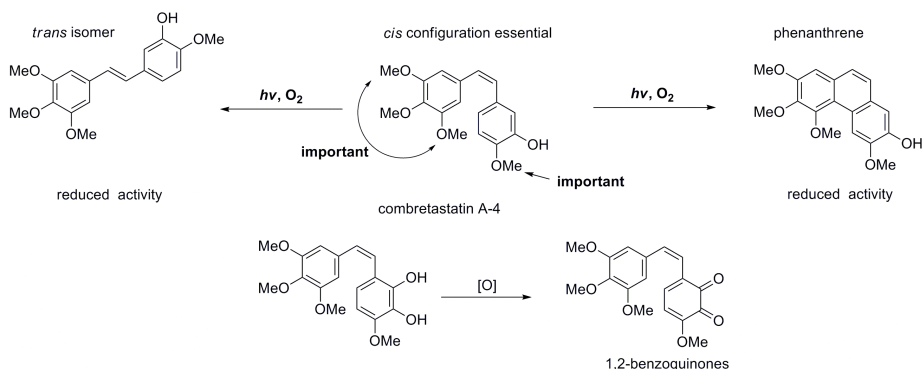


Figure 8 Combretastatins can isomerize to the more stable *trans*-configuration, and also form phenantrenes. The combretastatin A-1 series can oxidise to 1,2-benzoquinones.

The *cis*-bond-substituent is important for activity, but is liable to isomerizes to the more stable, but less potent *trans*-configuration.[9] It has therefore been proposed that a forced *cis*-bond structure might show higher stability than a free double bond arrangement, with several cyclic double-bond substituent's to restrict and lock the *cis*-configuration among the plausible solutions. The synthetic routes should also be highly stereoselective in respect to the difficult chromatographic separation of the two *Z/E*- stilbenes.[10] A cyclic moiety that both preserve the integrity and stability of the *cis*-isomers are the heterocyclic 1,2,3-triazoles available through thermic or catalytically driven cycloadditions. The derivatives can be further diversified by the positioning of the B-ring 1,4- or 1,5 respectively. Triazoles as *cis*-restricting linker group affords several advantages compared to the more simple stilbenes as it is highly stable towards chemical and metabolic transformation, such as oxidation and reduction, and withstand both acidic and basic hydrolysis.

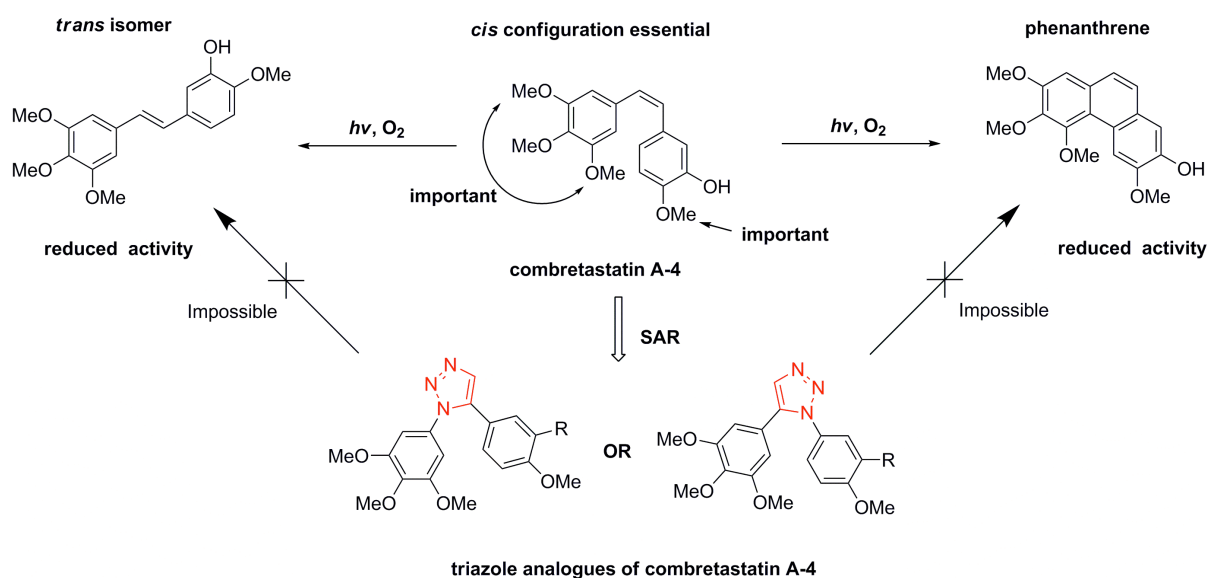


Figure 9 Triazole linker as *cis*-restricting moiety increases stability

Previous work with 1,4 or 1,5- disubstituted 1,2,3-triazoles as *cis*-restricting moieties have been performed by Odlo and Hansen [11-13], and forms the fundamental approach towards the synthesis of new derivatives as followed.

1.9. Synthesis of triazoles

The traditional route to achieve the triazole moiety is by the thermal Huisgen cycloaddition of azide and alkyne. The reaction yields both regioisomers with a subsequent challenging chromatographic separation. The regioisomer 1,4-diaryl-1,2,3-triazoles can be selectively obtained from azides and alkynes by use of a copper-catalyzed cycloaddition reaction, whereas the corresponding 1,5-diaryl-1,2,3-triazoles by either Grignard reagents or ruthenium catalysis. A new method have been reported for selective synthesis of 1,5-triazole analogues involving *tetra*-alkyl ammonium hydroxide.

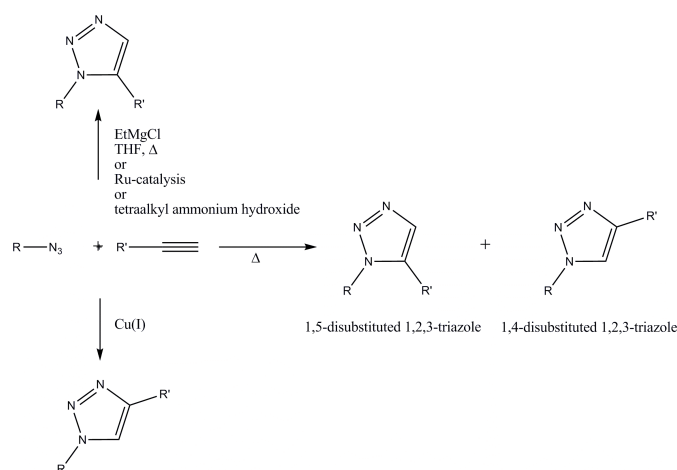


Figure 10 Routes to achieve 1,2,3- triazoles

1.10. Huisgen azide-alkyne cycloaddition reaction

The thermal Huisgen *A*zide-*A*lkyne *C*ycloaddition (AAC) method is probably one of, or the most useful member of the Huisgen 1,3-dipolar cycloaddition family. It is an un-catalyzed reaction requiring elevated temperatures for prolonged periods of time, and results in both 1,4- and 1,5- isomers being formed.

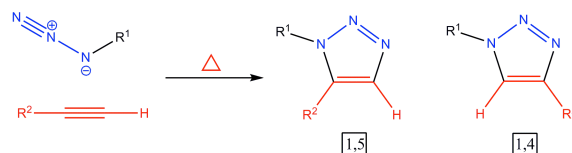


Figure 11 Thermal Huisgen Cycloaddition reaction is non-regiospecific

The mechanism involves the dipolarophilic alkyne reacting with a 1,3-dipolar azide to form a 5-membered heterocycle. Isomeric ratio can be altered somewhat with electron-withdrawing groups on the acetylene favouring formation of the 1,4-isomer, and electron withdrawing groups on the azide favouring formation of the 1,5- isomer. Chromatographic separation is therefore required to attain the two isomers, and may prove challenging due to the close chemical and physical resemblance. [14]

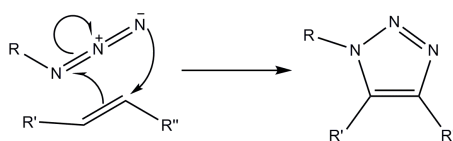


Figure 12 The mechanism of the Huisgen 1,3-Dipolar Cycloaddition represented by an azide and alkyne-dipolarophile.

1.11. Copper(I) catalyzed azide-alkyne cycloaddition; CuAAC

The copper(I) catalyzed azide-alkyne cycloaddition is a highly regiospecific reaction forming the 1,4 disubstituted 1,2,3-triazoles in excellent yield. The method is fast and straightforward, tolerates a broad range of functional groups, operates at a pH-range from 4 to 12 (although most are run at neutral pH), and is run in benign media such as aqueous *tert*-butanol or ethanol, or water without organic co-solvent. The reaction usually proceeds to completion within 6 to 36 hours at room temperature. It is often referred to as a “Click”-chemistry reaction, a term defined by Sharpless in 2002.

The procedure is simple and straight-forward; the azide and terminal alkyne are mixed in an aqueous solution of *tert*-butanol (or ethanol) and water, sodium ascorbate (5-10 mol%, frequently 10 mol%), and a copper(II)sulfate pentahydrate solution (1-5 mol%, frequently 5 mol%). The vial is sealed and vigorously stirred at room temperature, and the final product precipitates out of solution after addition of water.

The copper catalyzed azide-alkyne cycloaddition (CuAAC) requires copper at oxidation state I+, which is best generated in situ by reduction of Cu(II) with an excess of sodium ascorbate.

Free Cu(I) is unstable in aqueous solution because of a process known as disproportionation. Cu(I) -ions can exchange electrons with one another to form unrecoverable Cu(0) and inactive Cu(II). In addition, Cu(I) complexes may react with oxygen to form Cu(II) and reactive oxygen species such as superoxide, peroxide, and hydroxyl radicals. To address this problem it has proven necessary to add a reducing agent generating Cu(I) from Cu(II), such as sodium ascorbate. Reducing Cu(II) salts in situ has proven the best source to Cu(I), and an excess of reducing agent is therefore used. Cu(II) salts is also less costly and often more pure than Cu(I) salts.

The use of Cu^I salts in aqueous systems directly, for example CuI, CuOTf • C₆H₆, and [Cu(NCCH₃)₄][PF₆], can be used in the absence of a reducing agent. The reaction usually require acetonitrile as co-solvent and one equivalent of a nitrogen base, for example triethylamine, 2,6- lutidine or pyridine. To minimize the formation of undesired by-products 2,6- lutidine should be used, as well as exclusion of oxygen to further improve product purity and yield. Residual copper can be removed by an ammonium hydroxide/citrate buffer, dithiocarbamate, or by washing with ethylenediamine tetraacetic acid (EDTA) solutions. A disadvantage to this reaction is that copper ions are known to be toxic to cells in micromolar concentrations, minimizing its use in cells and living organisms.

A proposed mechanism for the catalytic cycle for the Cu^I-catalyzed ligation has been described by Sharpless and Rostovtsev, but the mechanism is still under investigation.

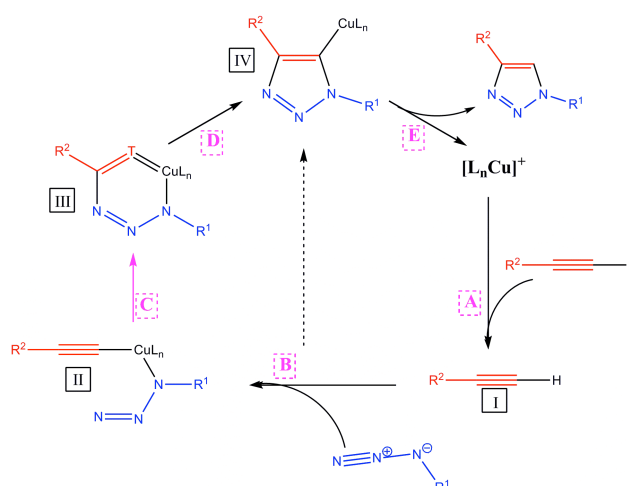


Figure 13 Proposed catalytic cycle for Cu(I)-catalyzed ligation

The proposed reaction mechanism begins with formation of the copper acetylide and proceeds via a six-membered copper-containing intermediate III. A recent analysis suggests

that both azide and alkyne are activated by the catalyst possibly within a multinuclear copper-acetylide species, which follows on from a report of two copper centres participating in the catalysis.

Organic solvent systems have found to be useful in polymer science, or where solubility problems arise, but will not be further discussed. [15]

1.12. Ruthenium catalyzed azide-alkyne cycloaddition; RuAAC

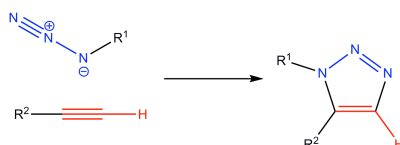


Figure 14 1,5-Disubstituted-1,2,3-triazole

A regioselective synthesis of 1,5- disubstituted-1,2,3-triazoles is provided by a ruthenium catalyzed cycloaddition of azides with both internal and terminal alkynes. The most efficient and regioselective catalyst are generally the [Cp*RuCl] complex. The reaction conditions require elevated temperatures through microwave irradiation, dry conditions, and an organic non-protic solvent such as benzene. [16] The synthesis is not applied in this thesis, and is not described in detail.

1.13. 1,5-diaryl 1,2,3-triazole synthesis using Grignard reagents

Formation of 1,5-diaryl 1,2,3-triazoles is achievable through use of Grignard reagents. The reagent is incompatible with several functional groups, and requires dry conditions to proceed. The method is not applied in this thesis and is therefore not described in detail.

1.14. An oxygen and moisture insensitive catalytic 1,5-diaryl-1,2,3-triazole reaction

A new approach to the regiospecific synthesis of disubstituted 1,5-triazoles have been proposed in an abstract of papers from a meeting held by the American Chemical Society. The method would supplement the thermally induced Huisgen cycloaddition, and the more selective 1,5-triazole forming Ru-catalyzed cycloaddition. The script consists of a two sentence text that roughly describes the terms and conditions for the synthesis, and reveal the results to be insensitive to both oxygen and moisture. The procedure allows for the regiospecific cycloaddition of an aryl azide and terminal alkyne in DMSO at room temperature, and with a catalytic amount of *tetra*-alkyl ammonium hydroxide at a

concentration of 10- 20 mol %. The method offers an appreciable simplification to the thermally induced counterparts, and would eliminate chromatographic separation of the isomers. Although the method is not described in detail the prospect of such a simplistic method sparked the interest for further investigations, in hope of detecting conditions with a plausible usefulness to the project.[17]

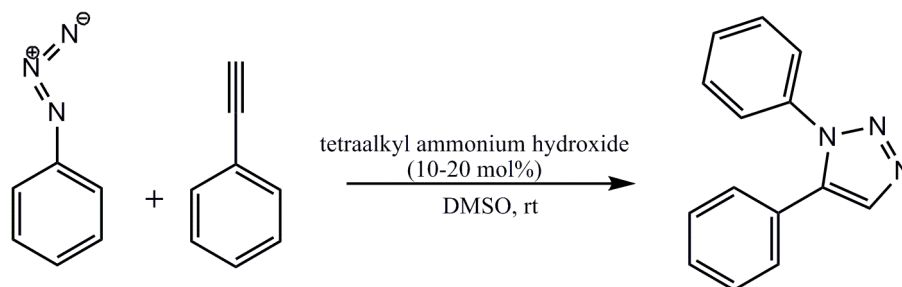


Figure 15 General reaction for metal-free catalytic synthesis of 1, 5-diaryl-1, 2, 3-triazoles

A search through commercially available *tetra*-alkyl ammonium hydroxide-moieties revealed that methyl, ethyl and up to *tetra*-butyl could be purchased as a crystalline solid or in a solution of water or methanol in differing molarities. Furthermore, the moieties included as candidates were narrowed down to stock-held chemicals already at the institute. 1-Azido-3,4,5-trimethoxyphenyl was used as nitrogen donating moiety procured from phenylboronic acid. 4-Ethynylanisole as acetylene-analogue afforded a simple scaffold resembling the *para*-substituted methoxy-group required in ring B of combretastatins.

1.15. Synthesis of aryl azides

The azide moiety is generally prepared *in situ*- prior to the AAC-reaction. There are several methods developed to generate alkyl azides, and a more limited selection regarding formation of aryl azides. The azide-generating method primarily used in this thesis utilizes phenylboronic azide, sodium azide and copper sulphate in methanol to generate the aryl azide.

Another method recently published reports the use of a new copper/diamine complex to yield alkyl azides from alkyl halides under mild reaction conditions. The method employs copper iodide or copper sulphate as Cu(I)-source reduced *in situ* by sodium ascorbate, stabilized by a ligand and in an aquatic solution under reflux and anaerobic conditions. The article aimed to optimize the Ullmann-type reaction which requires rather high reaction temperatures and is hampered by low reaction rates. There are several factors known to influence the reaction rate, such as choice of ligand and solvent system. As such the copper/diamine complex was proved to increase the efficiency to such a degree that traditional heating proved equally

efficient as microwave-irradiation, substantially simplifying the method.[18, 19] Among the ligands reported to accelerate the reaction were (1*S*,2*S*)-*N*¹,*N*²-dimethylcyclohexane-1,2-diamine (ligand d) which offered shorter reaction time in comparison with several other nitrogen containing ligands.

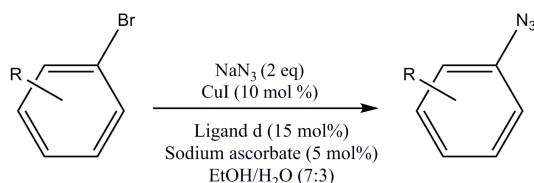


Figure 16 Reaction conditions for converting aryl halides with ligand d

The interest in this method is linked to the limited and few methods available regarding the conversion of aryl halides versus alkyl halides [20]. The reaction involves CuI or CuSO₄ as Cu(I)–source as mentioned, with Sodium ascorbate to stabilize and reduce an eventual oxidation of the catalyst. The role of the ligand is to form a Cu(I)/diamine catalyst complex, further stabilized by sodium ascorbate. Different solvent systems were screened with EtOH-H₂O (7:3) as the best choice, and *tert*-BuOH- H₂O (2:19) as another suitable medium. Microwave-irradiation could be replaced with traditional heating with these conditions, under an argon atmosphere presumably to prevent oxygen-induced Cu-oxidation.[21]

Several aryl bromides were located and included in the potential reaction repertoire. Inclusion criteria were the analogues ready availability, and similarity to the combretastatin A-series required *para*-methoxy group. It was also of interest to explore the reactions sensitivity to steric hindrance as well as electronic effects.

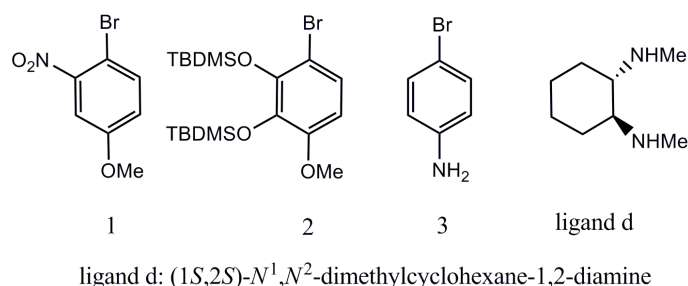


Figure 17 Potential arylbromide analogues

The new reaction offers a promising and interesting angle to azide-preparation with regards to a possible one-pot procedure with the copper catalyzed 1,4-diaryl-1,2,3-triazoles in this thesis. [22] Several advantages arise from a reduced number of synthetic steps such as reduced loss of product during reaction work-up and handling, as well as time spent on these operations, and minimized exposure to potentially carcinogenic and harmful chemicals.

As such the reaction conditions show close resemblance to the conditions yielding the desired 1,4-disubstituted-1,2,3-triazole from azides and terminal acetylenes, and the method was postulated to span both these reactions in a one-pot two-step synthesis from arylbromide to azide followed by CuAAC yielding the designated triazole. The Cu-catalyzed cycloaddition of aryl-azides with terminal alkynes reports a slightly higher catalyst and reductive agent loadings, but is other vice similar in conditions.

As model substances for the one-pot procedure the availability of compounds determined the analogues chosen. As the alkyne moiety it was also a criterion that it should be simple, and resemble the B-ring of CA-1. The best analogue available turned out to be 4-ethynylanisole. The bromide-analogues would be the same as those conveyed to azides in the previous chapter.

1.16. Synthesis of alkynes

Two reported routes towards the synthesis of internal alkynes are the Sonogashira coupling reaction between a terminal alkyne and aryl or vinyl halide. The Colvin rearrangement is a method for the synthesis of terminal alkynes starting from aldehydes.

The Sonogashira reaction couples terminal alkynes with aryl or vinyl halides, requiring a palladium catalyst, a copper(I)-cocatalyst and an amine base under anhydrous and anaerobic conditions to perform. Reactivity of the halide-moiety is proportional to its electro-negativity, and bromide analogues with electro-negativity of 2.8 respectively should therefore react in this coupling. Higher reactivity could be achieved by conversion to the more electronegative iodide moiety if necessary.

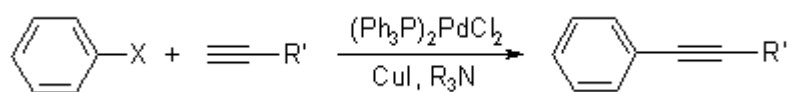


Figure 18 Sonogashira Coupling reaction conditions

The Colvin rearrangement forms terminal acetylenes from aldehydes by aid of lithium diisopropylamide and TMS-diazomethane, and the proposed synthesis involves protection of the catechol moiety as a methoxymethyl-ether.

2. Results and discussion

The first route to the alkyne-moiety was postulated to afford a TBDMS-protected alkyne amenable to undergo a Sonogashira-reaction yielding the corresponding triazole as depicted in the figure. The Sonogashira reaction was shortly described in the introduction.

Tert-butyldimethylsilyloxy is a protective group suited to shield the catechol from degradation or incompatible reaction conditions, and constituted the next step to yield 1-bromo-2,3bis(*TBDMS*)-4-methoxybenzene. The TBDMS-group would constitute a new tool to the growing synthetic library of combretastatins, and had not been performed earlier. Deprotection is readily achieved under acidic conditions to reveal the catechol. ^1H NMR signals are dedicated with the aromatic protons at δ 7.04 and 6.39 respectively, the *para*-methoxy group at δ 3.72, and the TBDMS-protons with *tert*-butyl at δ 1.00 and dimethyl at δ 0.12.

The synthesis of 1-((trimethylsilyl)ethynyl)-2,3bis(*TBDMS*)-4-methoxybenzene was performed in a Sonogashira reaction as depicted earlier.[23, 24] Trimethylsilyl acetylene as the alkyne-moiety would prevent polymerization of the product into a bi-arylic compound, and necessitates removal of the trimethylsilyl group before CuAAC. The ^1H - NMR spectra was additionally supported by ^{13}C -NMR to reveal the characteristic alkyne-signals in the carbon spectrum. Evident by the lack of signals in the alkyne region the reaction failed in forming the product with the proposed synthesis as viewed in the ^{13}C -spectrum. The reaction was performed twice on a 1 mmol scale yielding 128 % (0.604 g) and 63 % (0.3 g) of product material. The first batch was evidently not completely dry when weighed.

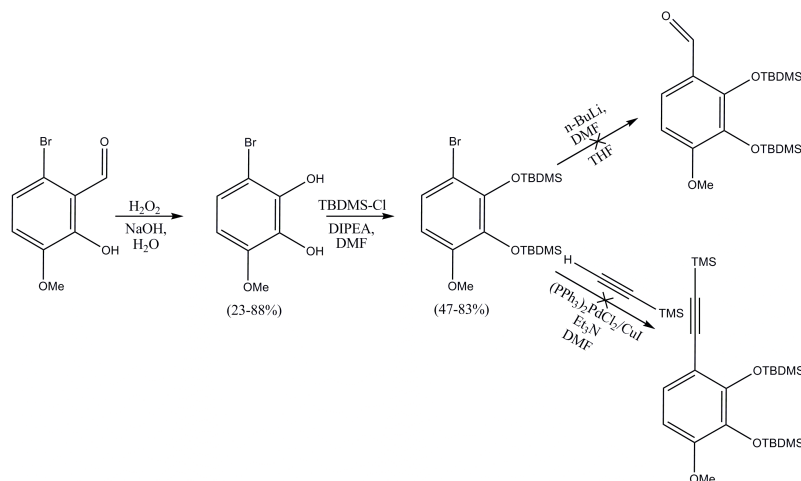


Figure 19 Synthesis of alkyne from aryl bromide with TBDMS-protected catechol

The bromide analogue was attempted converted to an aldehyde with *n*-Butyl lithium in tetrahydrofuran, and dimethylformamide, as to achieve the alkyne-moiety through a subsequent Colvin rearrangement. The reaction did not perform according to either ^1H -NMR nor ^{13}C -NMR, as signals from the aldehyde proton is not present.

The synthesis of the 1,4-disubstituted triazole was therefore achieved through another route integrating the aldehyde from the start of synthesis, and achieves the alkyne-moiety by the Colvin rearrangement of the aromatic aldehyde.

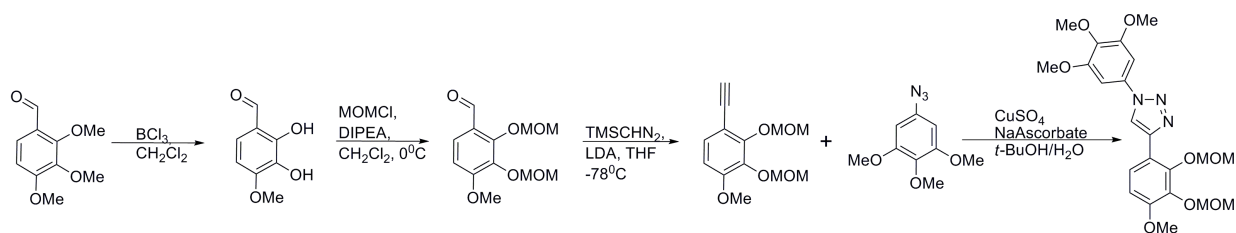


Figure 20 Synthesis of 1,4-triazole with alkyne substituted with methoxymethyl ether-protected catechol

The start of this is a Dakin's reaction which selectively demethylates 2-hydroxy-3-methoxy-4-methoxybenzaldehyde. The aryl aldehyde is oxidized by hydrogen peroxide in the presence of a base to afford the phenol. The aryl formate produced as an intermediate is subsequently saponified to yield the phenol, and the reaction is further favoured by a phenol- or amine substituent present at the *ortho*- or *para*-position. As such the reaction performed well to yield the unstable catechol 1-bromo-2,3-dihydroxy-4-methoxybenzene.

The method used in this step for the Dakin reaction was adapted from an article with slight modifications.[25] Addition of hydrogen peroxide was performed twice with a two hour delay, which could be reduced to a single addition according to a halt in reaction-development evident by TLC. The aqueous workup of this method includes acidification to

afford a protic environment for the catechol, and neutralization of hydrogen peroxide with sodium-thiosulfate to afford water. The yields were substantially increased when reaction time was shortened down to a minimum, apparent by TLC not conveying further product formation. The original article reports purifying the product by recrystallization from methanol, but this was not achieved in the lab as both cold and warm methanol dissolved the product. Without further purification the product was taken directly to the next protective step as NMR-analysis showed low or no possible interfering residuals from the reaction materials, especially the lack of aldehyde proton signal. The reaction was run several times, and the low yields during these runs may be due to the mentioned unstable nature of the catechols.

^1H NMR of the product reveals no trace of the aldehyde-proton occurring in the δ 11-11.5 ppm region, with the two aromatic protons at δ 6.96 and δ 6.40, the phenolic protons reported as a single signal rather than two distinguishable singlets at δ 5.51, and the *para*-methoxy group at δ 3.86.

The selective demethylation of 2,3,4-trimethoxybenzaldehyde was achieved through a Dakins reaction affording the 2,3-dihydroxy-4-methoxybenzaldehyde.[25] ^1H NMR reveals a strong signal at δ 11.08 dedicated the aldehyde-proton, the aromatic protons at 7.11 ppm and 6.59 ppm, and the catechol protons are distinguishable by a possible hydrogen-bond between the aldehyde and the *ortho*-positioned phenol resulting in a highly deshielded signal at 9.73 ppm, with the second phenol at 5.48 ppm. The *para*-methoxy group is evident at 3.96 ppm. In accordance to the unstable nature of the catechol the product was taken directly to the next step without further purification.

Protection of 2,3-dihydroxy-4-methoxybenzaldehyde was performed with methoxymethyl chloride (MOM-Cl), a protective group which performs well with phenols and is easily removed in acidic environment. [26]

^1H NMR reveals the aldehyde-proton at δ 10.25 and loss of signal from the *ortho*-positioned phenolic proton which would have appeared around 9 ppm and evident in 2,3-Dihydroxy-4-methoxybenzaldehyde. The aromatic protons appear at 7.6 and 6.78 ppm with the characteristic *para*- methoxy singlet at 3.52. The MOM-protection groups appear with the signals at 5.24 and 5.1 ppm for the $-\text{CH}_2$ and 3.89 and 3.58 ppm for $-\text{CH}_3$ respectively.

The MOM-protected aldehyde was successfully converted to 1-ethynyl- 2,3-bis(methoxymethoxy)-4-methoxybenzene in low to moderate yield. The product is identified

by its characteristic alkyne-proton at 3.17 ppm in the ^1H NMR-spectra. Residing signals are as dedicated in the MOM-protected aldehyde. This constitutes the last step in the synthesis before coupling with the 5-azido-1,2,3,-trimethoxybenzene and CuAAC. Product formation was further verified by ^{13}C -NMR to reveal the characteristic alkyne signals in the ~ 3 ppm region.

The successive connection of the two aryl moieties were performed with the regioselective Cu-catalyzed aromatic addition reaction yielding the 1,4-disubstituted triazole. Two analogues were synthesized according to the method which afforded 4-(4-methoxy-2,3-bis(methoxymethoxy)phenyl)-1-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazole in high yields ($\sim 80\%$). The catechol moiety in the triazole has proven unstable in the laboratory, and has therefore been stored as the methoxymethyl-protected analogue. The method used for deprotection was suboptimal, yielding both protected and unprotected triazoles. This might be due to the process of deprotection taking longer time than expected. Chromatographic separation was required, and it became evident from the presence of aldehyde signals that the eluent should contain a protic element to prevent oxidation. It is therefore suggested addition of 1 % acetic acid to the eluent, and use of deactivated silica when performing flash-chromatography.

The combretastatin A-1 series with its catechol moieties are preferentially deprotected just prior to biological testing to avoid degradation, or stored chilled in freezer with nitrogen atmosphere before further functional group interconversions are performed.

2.1. The copper/diamine catalyzed synthesis of aryl azides from aryl halides

The synthesis of the CA-1 derivatives was conducted with the nitrogen-donating azide on the A-ring of the molecule, and the acetylene-derivative on the B-ring. Switching the nitrogen-donating moiety to the B-ring would give isomeric derivatives with different chemo-physical properties, also of interest regarding SAR-elucidation. Such modifications will highlight the role of electronegative charges and its regiospecific placement within the triazol linker. The triazole moiety was constructed through the aromatic cycloaddition of azides and terminal acetylenes with copper catalysis, a method that regiospecifically construct 1,4-disubstituted triazoles in high yield. With the aid of a newly reported method[22] the azide-moiety were derived from aromatic bromides in a one step procedure. The method has shown to convert steric unhindered aromatic bromides into azides in varying degree of success depending on

the electronic influence within the molecule. The successful conversion of aryl bromides with a simple and mild reaction would increase the availability of azide-moieties greatly.

Three potential arylbromide analogues were chosen for this experiment by the simple criteria of ready availability in the chemical supply library. As such the reaction was explored with three bromide- analogs;

- 1) Reactivity vs electron-withdrawing nitrogroup: 1-bromo-4-methoxy-2-nitrobenzene, an incompatible functional group with Grignard reagents and therefore do not yield 1,5-triazoles without synthetic interventions.
- 2) Sterically hindered Br with TBDMS-protected catechols: 3-bromo-6-methoxy-1,2-phenylenebis(oxy)bis(*tert*-butyldimethylsilane, previously synthesized in the laboratory, and
- 3) 4-bromoaniline

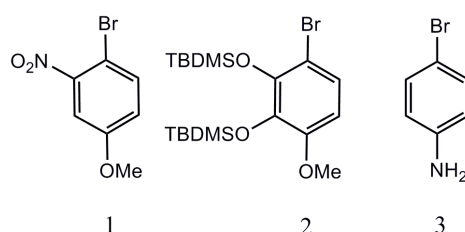


Figure 21 Potential arylbromide analogs

As such the method was conducted with an electron-withdrawing nitro group *ortho*-positioned to the bromide, an electron-donating amino-group in *para*-position, and a TBDMS-protected catechol as a steric hindering moiety. The reactions were not intended to mark the compatibility of the method with different moieties, a process which would span a wider array of analogues, but were more a tentative test of simple aryl-bromides ability to yield azides through this method. Thus the analogues were chosen according to resemblance to the A-ring in the CA-series and availability in stock. Of the three moieties the nitro-substituted molecule 1-bromo-4-methoxy-2-nitrobenzene gave a strong-colored red oil in quantitative yields, which were easily traced during flash-chromatography affording 1-azido-4-methoxy-2-nitrobenzene in low yields (7%). This product was further identified by IR-analysis which stated the characteristic azide-absorption band which appears approximately $\sim 2100\text{--}2300\text{ cm}^{-1}$. The amine 4-bromoaniline gave no product according to the chosen TLC-system, but did develop an increasingly darker brown coloration of the starting material in the reaction mixture portrayed by TLC. The failed reaction can thus be caused either by the influence of amine in *para*-position affecting reactivity of the halide, or by thermal decomposition of the starting material during reflux. The TBDMS-protected bromide 1-bromo-2,3-bis(*tert*butyldimethylsilyloxy)-4-methoxybenzene gave a

heterogeneous white and green waxy solid, which proved to be un-reacted bromide and polymerized material unsolvable in EtOAc, CDCl_3 , hexane and DMSO. The solvent medium used in this method is not optimal for the TBDMS-protected arylbromide as solvation took several hours to achieve.

The close resemblance between the arylbromide converting method and the CuAAC make it liable for further modifications. Both utilize sodium ascorbate to reduce Cu(II) to the reactive Cu(I)-species, although in slightly different mol-percents. As noted earlier the concentration of Cu may affect the reaction according to the quantitative formation of different Cu-clusters, a formation sensitive to a wide array of different factors present in the medium. The identities of the more catalytically effective cluster-species are unknown, as well as the mechanisms for equilibrium and the optimal conditions for their formation. The two methods were merged in an attempt at a one-pot reaction by adopting the conditions according to the highest molar concentrations involved among the two reactions, and including the molar concentrations of reagents found exclusively in one of the reactions. Thus the new reactants included ligand **d** and sodium azide from the arylbromide conversion reaction, and a higher concentration of CuI and the solvent-system was adopted from CuAAC. A discrepancy in type of solvent altering between *tert*-butanol/ water (1:1) and ethanol/water (7:3) is due to *tert*-butanols high melting-point, and which constantly froze solid in the laboratory during winter. To avoid this in later reactions a larger volume of the solvent-mixture were prepared and stored. The optimal solvent-system was not tested, as the CuAAC performs well in several aqueous alcoholic media, but its effect on the aryl bromide converting reaction remains uncharted. Reactions performed as one-pot reactions were first tested on two simple components to assess its usability. The nitro-substituted bromide and the simple *para*-substituted ethynylanisole were merged in relatively low yield as test-substrates, but the synthesis was usually performed within 15 minutes, though left longer to assure no further development. The successive work-up was performed quickly by adding a 10 % aqueous ammonia-solution to remove traces of copper, stirred and another addition of water to increase the volume. The precipitation of the lipophilic product was followed by simple filtration.

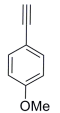
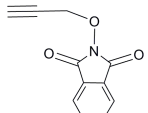
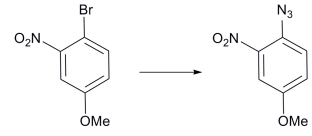
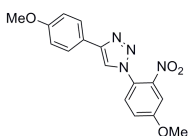
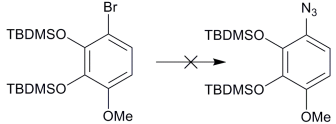
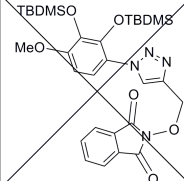
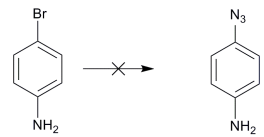
		
		X not attempted
	X not attempted	
	X not attempted	X not attempted
Triazole conversion with ligand d		One pot procedure yielding 1,4-diaryl-1,2,3-triazole

Figure 22 Table of required azide analogues and attempted one-pot reactions

2.2. One-pot two-step Cu-catalyzed azide-alkyne cycloaddition reaction with ligand d

Two reactions were attempted according to the one-pot two-step procedure where the cycloaddition between 4-bromo-3-nitroanisole and 1-ethynyl-anisole afforded 1-(4-methoxy-2-nitrophenyl)-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole in low yield (7%). The product displays the characteristic 1,4-triazole signal at ~8 ppm, but was unfortunately not analyzed by ¹³C-NMR.

The reaction between 1-bromo-2,3 bis(*TBDMS*)-4-methoxybenzene and *n*-(proparguloxo)-phthalamide did not form a triazole. TLC-analysis did not reveal any conversion of the starting materials, and the reaction evolved motley green and white crystals. This was confirmed to be unreacted arylbromide with TLC, and probable polymerized material due to its insolubility in ethyl acetate, dimethylsulfoxide and chloroform. A table of the attempted reactions can be viewed in figure 22.

The reactants to molecule 2-((1-(2,3-bis(*TBDMS*)-4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)isoindoline-1,3-dione were chosen to discern whether the two carbon-linker in the acetylene-donating molecule could attribute positively to the reactions reactivity. As such the acetylene-moiety was chosen as a readily available dienophile, with its two carbon-link to the more bulky phthalamide moiety. The results from the azide-forming conversion of the

arylbromide used in this reaction had not yet been clarified, which coloration seemed promising in regards of product-formation. The negative results from the azide-forming reaction would probably have proven a discarding factor to the new attempt. The reaction involves two bulky reactions, which if successful would insinuate that less bulky constituents would react in the same reaction. The two carbon linker between phthalamide and the acetylene increases rotational capacity, and therefore thought to enable the acetylene to react to the azide without steric hindrance from the phthalamide. This was not achieved with this reaction.

2.3. An oxygen and moisture insensitive 1,5-disubstituted-1,2,3-triazole reaction

The reported method of conveying 1,5-diaryl-1,2,3-triazoles was found interesting to this project. As such the *tetra*-alkyl ammonium hydroxide moieties are obtainable as crystalline solids or as aquatic or methanol- containing solutions of different molarities. The results achieved are therefore correlated to two variables, the base moiety and its physical state. Drawing a parallel to the relevance of forming the proper catalytically active copper-clusters in CuAAC [15, 21, 27], the solution media might play a role in the unknown mechanistic conditions required for this reaction to perform. The experiments performed with *tetra*-methyl and *tetra*-butyl-moieties can therefore stem from slow-reacting or improper bases, or as influenced negatively by the media.

The results obtained from the reactions clearly point *tetra*-ethyl ammonium hydroxide (25 % in H₂O) as the best moiety for further reactions.

Each analogue of the *tetra*-alkyl ammonium hydroxides were tested in a reaction with a catalytical loading of 20 mol % and 1 equivalent respectively. This did not discern whether the catalytical loading would influence the reaction, but could eliminate some complications due to the old age of the *tetra*-ethyl and *tetra*-butyl analogues. Exact yields for the reactions are not obtainable, though a rough indication of the reactions performance can be gained from the following:

Rough reaction yields from the following *tetra*-alkyl ammonium hydroxides are as follows:

Tetra-methyl ammonium hydroxide (25% in MeOH) < 5 % yields

Tetra-ethyl ammonium hydroxide (25 % in H₂O) 34- 60 % yield

Tetra-butyl ammonium hydroxide (25% in MeOH) ~ 6 %

The experiments with *tetra*-methyl ammonium hydroxide (25% in MeOH) did afford the desired 1,5-disubstituted triazole as a precipitate in very low yields as stated by ^1H -NMR. The *tetra*-butyl ammonium hydroxide (25% in MeOH) were not attempted precipitated but extracted directly and the resulting product solved in EtOAc before partitioning with hexane. The resulting precipitate was analysed on NMR without further purification to verify possible triazole formation in low yield. The spectrum contains signals from the desired triazole in low yields, though additional signals from impurities are considerably stronger. The low product formation does not favour these analogues for further experiments. The two *tetra*-alkyl analogues are both a 25% solution in MeOH, which is a variable not further explored.

To fully explore the potential of solubilising effects of MeOH versus aqueous solutions in the sample the procedure could have been performed with solid analogues of the *tetra*-alkyl ammonium hydroxide. These were not obtainable at the present, and the various solutions were therefore dried on a vacuum-line for two days to obtain the dry ammonium hydroxide analogues. If use of resulting dry base increased the yields of *tetra*-methyl and *tetra*-butyl, whose yields were low, then the physical state of the base, or solution medium, might affect the reaction. The *tetra*-ethyl-moiety were tested as dry substance and resolved as 25 % in MeOH, but this would not elude whether or not the yields were caused by the nature of base or its physical state. It was however not possible to obtain results from the two other base-moieties, as the limiting factor at this point proved to be the pre-synthesized azide moiety.

Another variable not investigated is the rate of formation as function of time, as all samples were left to stir for 1-2 days. It can be difficult to distinguish a slow reaction from a halted conversion when the ratio between starting materials and formed triazole is high with TLC-analysis. This was not further addressed in the experiment as the catalytic ability with this procedure became relative between the three analogues, and time did not allow further studies.

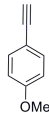
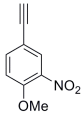
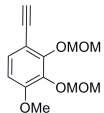
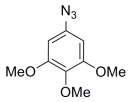
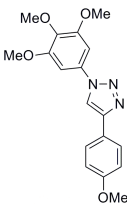
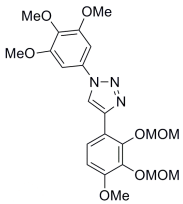
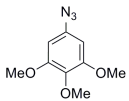
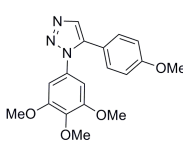
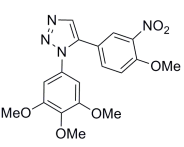
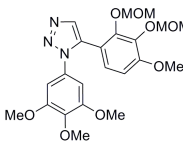
<div>Alkyne-moieties</div> <div>Azide- moiety</div>	 OMe	 OMe NO ₂	 OMOM OMOM OMe
 1,4-Triazoles formation through CuAAC	 OMe	Not attempted	 OMOM OMOM OMe
 1,5-triazole formation through tetraethyl ammonium hydroxide reaction	 OMe	 OMe NO ₂	 OMOM OMOM OMe

Figure 23 Analogues achieved with CuAAC (middle row) and the tetraethyl ammonium hydroxide reaction (bottom row).

The method with *tetra*-ethyl ammonium hydroxide (25 % in H₂O) formed the 1,5-disubstituted triazole analogue 5-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole under mild conditions and minimal workup. In comparison with the more demanding Ru-AAC, Grignard reactions and the thermal Azide-Alkyne Cycloaddition-reaction, the simplicity is profoundly the major advantage to this method. The scope of compatible reactants is uncharted, as well as optimal reaction conditions and true nature of the base, all factors that the upcoming article from Scripps Institute most certainly covers. Until the complete method is revealed the reaction elucidated here may be worth a try. In cases where the more complicated reactants fail to react under the noted terms and conditions, retrieving the reactants from the reaction-mixture is achievable by liquid-extraction followed by chromatography.

The ¹H-NMR-specter of 5-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole states a clear 1, 5-triazole proton signal at 7.80 ppm, which appears 8.04 in the corresponding 1, 4-triazole analogue 4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole which was synthesized for comparison reasons. A relatively small shift of about ~ 0,24 ppm, can be further established as the regioisomeric difference between the two triazoles if synthesizing the 1,5-triazole through ruthenium-catalyzed azide-alkyne cycloaddition (RuAAC) or with magnesium catalyst. Thus the exact ppm values of both triazole-proton alignments in the ¹H-NMR spectre could be compared to the new method, and exclude any uncertainty regarding this specific 1,2,3-triazoles true isomeric nature. The Ru-AAC-reaction

requires anaerobe, dry conditions and microwave-irradiation to perform, or can experimentally be performed by Grignard reagents or the thermal Huisgen AAC. This is a procedure investigated as part of another master-thesis at the faculty, but which results has not yet been retrievable. The final evaluation of the identity of compounds by this method is therefore not eluded until compared to a known 1,5-triazole by either Ru-AAC, Grignard or thermal Huisgen cycloaddition which yields both regioisomers and requires a difficult chromatographic separation. The thermal AAC might therefore not be suited for this task as separation is challenging, and also may afford the 1,5-regioisomer in low yields.

Method development of metal-free catalytic 1,5-disubstituted-1,2,3-triazole reaction, and sample work-up:

The reaction conditions were simply stated as azide, acetylene-moiety, a *tetra*-alkyl ammonium hydroxide analogue (10-20 mol %) in DMSO at ambient temperature. Prior to the first reaction it was anticipated that precipitation might occur upon addition of water. DMSO is a highly polar aprotic solvent miscible in water and a wide range of organic solvents, and its dilution with water was therefore thought to lead to precipitation of the lipophile triazole followed by filtration. Addition of water was first attempted to break the solubilising effect, but this did not cause precipitation. The mixture was therefore extracted with EtOAc, washed with brine to remove salts, and again with water to remove traces of brine and DMSO. Upon resolving the oil in eluent (Hex: EtOAc 4:1) for flash chromatography precipitation occurred that could be filtrated with a standard folded filter and left to dry. The resulting beige powder was tested on NMR to reveal a single regioisomer of 1,5-disubstituted-1,2,3-triazoles in 34 % yield. This first run proved the method promising, and subsequent reactions with different *tetra*-alkyl ammonium bases were planned.

Three compounds were attempted synthesized by the method using *tetra*-ethyl ammonium hydroxide (25% in H₂O); thus affording the three moieties in the bottom line in figure 23
5-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole,
5-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole and
5-(4-Methoxy-2,3-bis(MOM)phenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole.

The compound 4-ethynyl-methoxy-2-nitrobenzene contains a *meta*-positioned nitro-group, incompatible with the Grignard reagents used for 1,5-disubstitutes triazole formation. It was therefore of interest to access its reactivity with the new method. As shown in the ¹H NMR spectra the 1,5-triazole proton and the *ortho*-positioned proton in ring B appear as two relatively close signals at 7.87 ppm and 7.82 ppm. This proton is one of the two *ortho*-

positioned protons relative to the triazole in ring B, with a high chemical shift due to the nitro-substituent and showing a coupling constant ($J=2$) towards its *meta*-positioned proton in the aromat at 7.35 which appears as a double doublet. This proton couples both at its *ortho* and *meta*-positioned proton neighbors, and identifying it as the second *meta*-positioned proton in ring B. The last proton in ring B appears at 7.05 ppm. The signals from ring A appears as a singlet due to molecular symmetry at 6.55 ppm, and the residual methoxy-groups constitute the remaining signals at 3.97 ppm (ring B), 3.85 ppm (para, ring A) and 3.73 ppm for the symmetrical *meta*-positioned methoxy-groups in ring A.

The 1,5-triazole (5-(4-methoxy-2,3-bis(MOM)phenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole) was synthesized for comparismental reasons to the corresponding 1,4- triazole with the same diaryl substituent's, and also potentially link the failed one-pot conversion of the TBDMS-protected bromide to steric hindrance including the MOM-group. The failed conversion of the aryl-bromide to azide prior to the cycloaddition in the adapted one-pot procedure could not elude whether steric hindrance was a limiting factor. The use of the MOM-protected analogue which has shown to perform well in the CuAAC-reaction would therefore be more helpful. The reaction mixture was attempted precipitated as previous samples by resolving the formed brown oil in a few drops EtOAc and 15 mL hexane. The large protecting methoxymethoxy-groups were proving to lipofilic to allow precipitation, which then was forced by diluting the mixture by adding 50 mL of water and stirring forcefully. The oil precipitated along the glass-surface and could therefore be dried and NMR-tested. According to the spectra both the formed 1,5-triazole indicated by its proton-signal at 7.82 ppm, and residual acetylene-starting material, indicated by the acetylene-proton at ~3 ppm, are present. A small peak at 8.55 ppm could indicate a 1,4-triazole analogue, but the ratio between the two is clearly very large. Extraction of product from the reaction mixture, followed by flash-chromatography, would be a better route to separate the substances in this particular reaction, as both the triazole and acetylene are hydrofobic enough to precipitate. The acetylene-derivative was the limiting factor to retry this reaction, and its time-consuming multistep synthesis did not allow for a new batch and re-run.

3. Future work and studies

The results obtained from the experiments in this thesis confirms the formation of selected 1,2,3-triazoles with low to moderate yields. The formation of 1,5-disubstituted triazoles was achieved through a mild moisture and oxygen insensitive reaction which were catalytically driven by *tetra*-ethyl ammonium hydroxide. The two other tetra-alkyl analogues can not be completely discarded as suitable catalysts, as the procedure during these experiments were both crude and tentative. Further optimalization of the reaction conditions, its work up and elucidation of catalytical analogues and physical state should be performed.

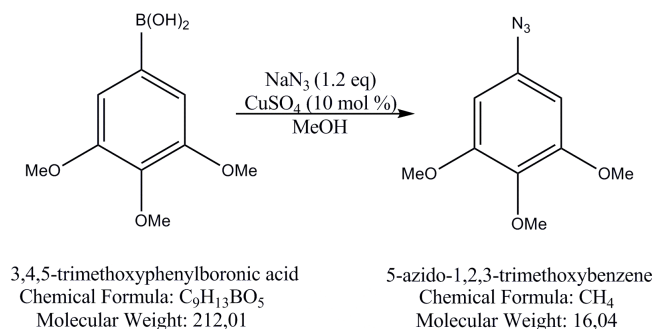
The method obtained by this thesis did afford a nitro-substituted triazole which would be difficult to attain by Grignard reagents.

The copper/diamine catalyzed synthesis of aryl azides from aryl halides performed relatively low yields, which could be further optimized. It did manage to convey 1,4-triazole formation in an adapted one-pot two-step reaction fusing both the aryl azide forming reaction with the more established copper catalyzed azide-alkyne cycloaddition.

Formation of the triazole-analogues was tentative, and yields attained did not allow for biological testing. As the methods may be optimized it is hoped that they may become useful tools in the triazole forming reaction kit, and confer new promising drug candidates.

4. Experimental

4.1. 5-Azido-1,2,3-trimethoxybenzene



Sodium azide (12 mmol, 0.78 g, 1.2 eq) and copper sulfate pentahydrate (1 mmol, 0.25 g, 10 mmol %) were placed in a round-bottom flask equipped with a stirring bar. Subsequently followed by addition of 3,4,5-trimethoxyphenylboronic acid (10 mmol, 2.12 g) and 15 mL MeOH. The reaction was stirred at ambient temperature for a minimum of 5 hours and monitored with TLC (Hex/EtOAc (2:1), R_f = 0.68), and quenched with 50 mL H_2O at completion, or no further conversion. The mixture was extracted with hexane (75 mL x 3), and the organic layer washed with 5 % aqueous NH_3 (50 mL x 2). The combined aqueous fraction were dried with anhydrous $MgSO_4$ before removal of the solvent in vacuo to give a pale orange solid. The product was purified with flash chromatography (silica 60_F, Hex: EtOAc 2:1) affording 5-azido-1,2,3-trimethoxybenzene as a yellow/orange solid.

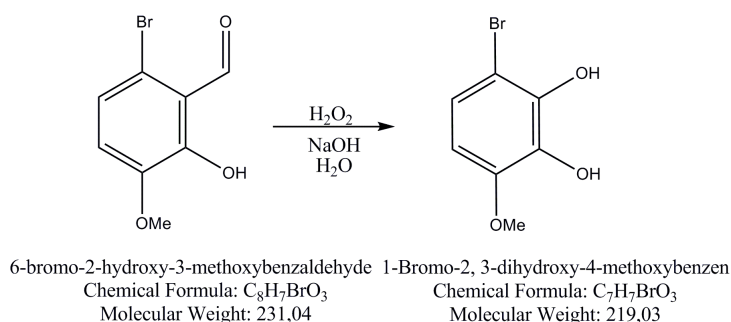
5-Azido-1,2,3-trimethoxybenzene; Orange solid, 32-87% yield,

1H NMR (300 MHz, $CDCl_3$) δ 6.22 (s, 2H), 3.82 (s, 6H), 3.78 (s, 3H).

TLC: R_f = 0.68 Hex/EtOAc (2:1).

4.2. 1-Bromo-2,3-dihydroxy-4-methoxybenzene

[28]



2-Hydroxy-3-methoxy-4-methoxybenzaldehyde (0.5 mmol, 1.16 g) was suspended in 2% sodium hydroxide (1.5 mL) in a one-necked round bottom flask equipped with a magnet bar. The suspension was cooled on ice-bath during drop wise addition of a 30% solution of hydrogen peroxide (0.14 mL, 2.8 eq). A color change from bright yellow to a brown/purple color was noted. The reaction mixture was monitored with TLC (Hex: EtOAc 2:1, $R_f = 0.35$), and acidified with 1 M HCl to pH 4-5(6) upon no further conversion by TLC, or when run to completion. The mixture was extracted with dichloromethane (20 mL x 3) and the organic fractions washed with 15% sodium thiosulfate to neutralize H_2O_2 , and the solvent dried with anhydrous $MgSO_4$ before removal in vacuo to afford 1-bromo-2,3-dihydroxy-4-methoxybenzen as a tan powder/solid.

The reaction was performed in several runs in 10 mmol scale (59.8% yield, 1.3 g, 24 hour run time), 0.5 mmol scale (28% yield, 31 mg, 74 h run time), 5 mmol scale (88% yield, 0.98 g, 2 h run time) and a 16 mmol scale (73 % yield, 2.5 g, 1.5 h run time).

1-Bromo-2,3-dihydroxy-4-methoxybenzene; tan powder/solid, 28-88 % yield,

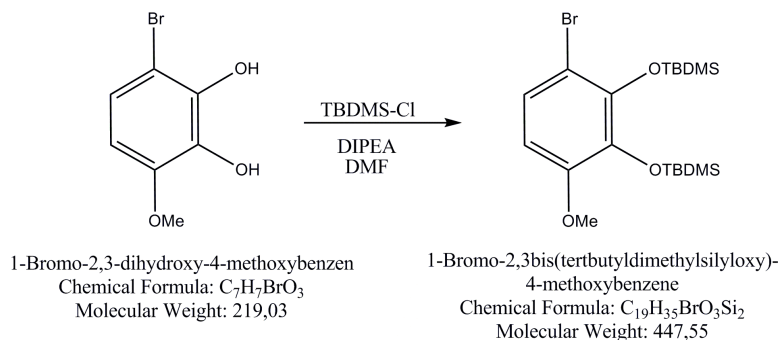
Mpoint: 115,4-119° C (Lit. 124°- 126° C),

1H NMR (300 MHz, $CDCl_3$) δ 6.96 (d, $J = 8.9$, 1H), 6.40 (d, $J = 8.9$, 1H), 5.51 (d, $J = 16.0$, 2H), 3.86 (s, 3H).

TLC: $R_f = 0.35$ Hex: EtOAc (2:1).

4.3. 1-Bromo-2,3bis(TBDMS)-4-methoxybenzene

[28]



Diisopropylpolyethylamine (18.6 mmol, 2.4 g, 3.1 eq) and *tert*-butyldimethylsilylchloride (12.5 mmol, 1.88 g, 2.2 eq) were added a solution of 1-Bromo-2,3-dihydroxy-methoxybenzene (6 mmol, 1.246 g) in dry dimethylformamide (25 mL) under nitrogen atmosphere and dry conditions. HCl evolution as smoke was noted upon addition of TBDMS-Cl. The mixture was left to stir at room temperature for about 3 h and monitored by TLC (Hex: EtOAc 10:1 R_f = 0.6). The reaction was terminated by addition of ice and extracted with dichloromethane (10 mL x 4) before the combined organic layers were washed with water (10 mL x 3), saturated sodium bicarbonate (15 mL x 3), water (15 mL), and dried with anhydrous $MgSO_4$. The solvent was removed in vacuo to give a brown oil. The product was purified with either flash- chromatography or with a molecular sieve consisting of celite, silica and anhydrous $MgSO_4$. Hex: EtOAc (10:1) was used as eluent, or to wash the sieve (400 mL). The solvent was removed in vacuo to afford 1-bromo-2,3 bis(*tert*-butyldimethylsilyloxy)-4-methoxybenzene as a white wax-like solid.

The reaction was performed in three scales with 6 mmol (62 %, 1.58 g), 4 mmol (39 %, 0.699 g) and 11 mmol (24.5 % 1.3 g).

1-Bromo-2,3bis(*tert*-butyldimethylsilyloxy)-4-methoxybenzene; White, waxy solid, 24,5-62 % yield, Mpoint: 53-57° C (Lit. Mpoint 68.9-69.9 ° C),

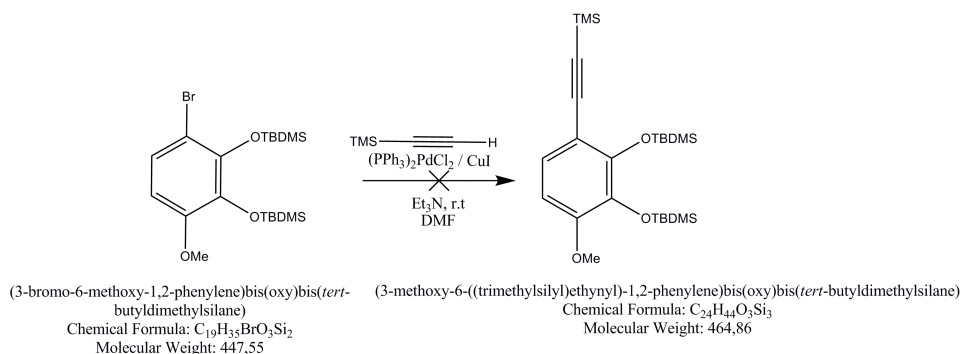
1H NMR (300 MHz, $CDCl_3$) δ 7.04 (d, J = 8.9, 1H), 6.39 (d, J = 8.9, 1H), 3.72 (s, 3H), 1.00 (d, 19H), 0.12 (d, 13H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 152.14, 145.71, 138.45, 124.71, 108.75, 106.04, 77.82, 77.60, 77.39, 76.97, 55.45, 26.85, 26.51, 19.15, 19.13, -2.70, -3.46.

TLC: R_f = 0.6 Hex: EtOAc (10:1)

4.4. 1-((Trimethylsilyl)ethynyl)-2,3bis(TBDMS)-4-methoxybenzene

[23, 24]



1-Bromo-2,3-bis(*tert*-butyldimethylsilyloxy)-4-methoxybenzene (0.46 g, 1 mmol) were introduced to dry dimethylformamide (5 mL) in a two-necked round-bottomed flask rigged to fit dry conditions, with reflux condenser and a magnetbar. Successively followed by addition of triethylamine (0.514 g, 5 equiv), Copper (I) iodide (0.0193 g, 10 mol %), bis(triphenylphosphine)palladium(II) chloride (0.14 g, 20 mol%) and trimethylsilyl acetylene (0.109 g, 1.11 equiv). The solution was heated to 160°C under reflux for about 24 h, and monitored by TLC (Hex: EtOAc 10:1, R_f = not obtainable). The reaction-mixture was filtered through a molecular sieve consisting of celite, silica and anhydrous MgSO₄ to remove polymerized residue, and washed with EtOAc (400 mL). Removal of the solvent in vacuo afforded a brown oil.

1-((Trimethylsilyl)ethynyl)-2,3bis(*tert*-butyldimethylsilyloxy)-4-methoxybenzene;

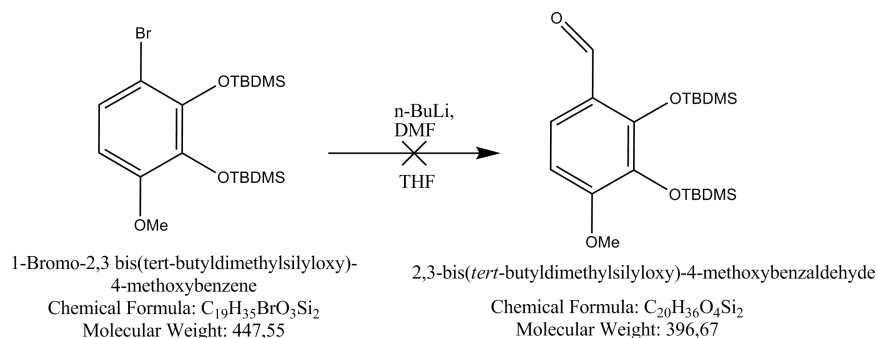
Brown oil, 75-151% yield,

¹H NMR (300 MHz, CDCl₃) δ 7.97 (s, 1H), 7.71 – 7.32 (m, 1H), 7.24 (s, 0H), 6.82 – 6.31 (m, 1H), 3.92 – 3.65 (m, 1H), 3.32 (s, 0H), 2.99 (dd, *J* = 22.0, 68.3, 6H), 1.26 – 0.76 (m, 1H), 0.27 – -0.10 (m, 1H),

¹³C NMR (75 MHz, CDCl₃) δ 163.04, 147.77, 144.83, 134.97, 134.83, 133.29, 132.86, 132.59, 132.56, 132.43, 131.47, 131.30, 131.13, 129.08, 128.92, 121.59, 119.97, 119.08, 113.18, 109.33, 108.27, 105.46, 104.00, 103.61, 77.86, 77.43, 77.01, 56.58, 56.51, 55.57, 36.93, 31.89, 26.41, 26.14, 26.05, 18.72, -3.92, -3.99.

TLC: R_f not obtainable, Hex: EtOAc (10:1).

4.5. 2,3-bis(TBDMS)-4-methoxybenzaldehyde



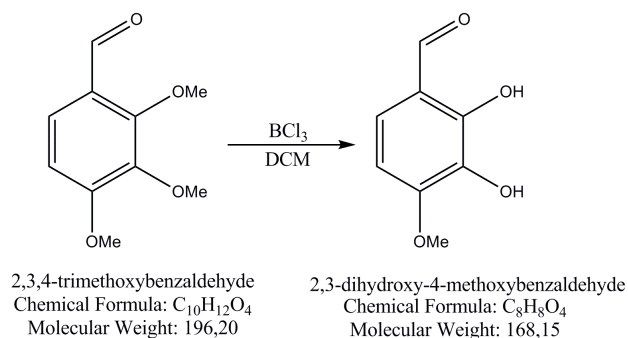
1-Bromo-2,3 bis(*tert*-butyldimethylsilyloxy)-4-methoxybenzene (1 mmol, 0.448g) and 5 mL Tetrahydrofuran (THF) were introduced to a two-necked round-bottomed flask rigged to fit dry conditions at - 78°C (dry ice/acetone bath). *n*-Butyl Lithium (1.6 M in hexanes)(1.2 equivalents) was added and the mixture stirred for 15-30 minutes. The mixture was added dimethylformamide (1.2 equivalents, 0.365 g, 0.385 mL) and allowed to stir at ambient temperature for 2-4 hours followed by TLC (Hex: EtOAc, 10:1) until no further development or the reaction was run to completion. The reaction was quenched with water (15 mL) and extracted with diethylether (3-5 mL). The combined organic fractions were dried with anhydrous MgSO₄ and the solvent removed in vacuo to afford a brown oil. The sample was tested on ¹H NMR to verify formation of the aldehyde before further purification. The analysis did not show the desired aldehyde signal and the reaction was rejected.

Product: dark brown oil.

¹H NMR (300 MHz, CDCl₃) δ 7.10 – 6.91 (m, 1H), 6.82 (d, *J* = 8.2, 0H), 6.52 – 6.30 (m, 2H), 5.45 (d, *J* = 11.3, 1H), 3.91 – 3.57 (m, 5H), 2.90 (d, *J* = 21.3, 1H), 1.54 (s, 1H), 1.41 (s, 1H), 1.24 (s, 1H), 1.11 – 0.72 (m, 28H), 0.33 – -0.06 (m, 19H).

4.6. 2,3-Dihydroxy-4-methoxybenzaldehyde

[25]



The aldehyde 2,3,4-trimethoxybenzaldehyde (1 mmol, 0.196 g,) was suspended in dry dichloromethane (3.1 mL) to give a yellow solution, under nitrogen atmosphere and ambient temperature, followed by the dropwise addition of borontrichloride (1.0 M in DCM, 1 mL, 1 eq). Evolution of smoke and change of color to a dark brown solution with red/green tint was noted. Another portion of borontrichloride (1.0 M in DCM, 1 mL, 1 eq) was added after 2 h, and the mixture left to stir over night. The reaction was monitored with TLC (Hex: EtOAc 1:2 m/TEA (2 drops), R_f = 0.55). Upon no further development, or when reaction was run to completion, the reaction was quenched by pouring the mixture into 10% NaHCO₃ with powerful gas-evolution. The aqueous layer was acidified with 1 M HCl (pH~ 1) and extracted with diethylether. The combined organic layers were dried with anhydrous MgSO₄, and the solvent removed in vacuo to give 2,3-dihydroxy-4-methoxybenzaldehyde as bottle green crystals.

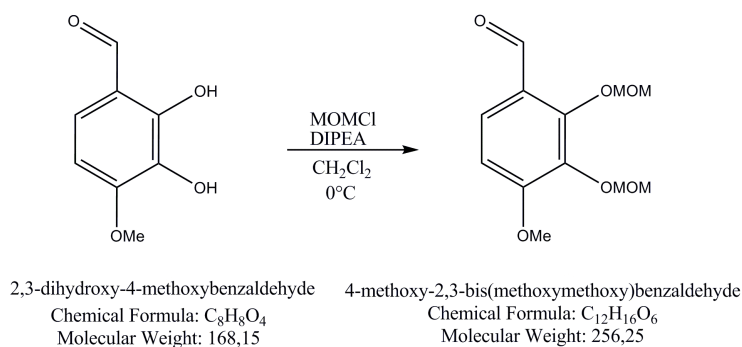
2,3-Dihydroxy-4-methoxybenzaldehyde; mossy green crystals, 110% yield (not entirely dry), M.point: 105,2 °C (lit 115-116°C),

¹H NMR (300 MHz, CDCl₃) δ 11.08 (s, 1H), 9.73 (s, 1H), 7.11 (d, *J* = 8.7, 1H), 6.59 (d, 1H), 5.48 (s, 1H), 3.96 (s, 3H).

TLC: R_f = 0.55 Hex:EtOAc:TEA (1:2: 2gtt), 0.75 Hex;EtOAc (1:2).

4.7. 4-Methoxy-2,3-bis(MOM)benzaldehyde

[29]



The aldehyde 2,3,4-trimethoxybenzaldehyde (1 mmol, 0.179 g) was dissolved in dry dichloromethane (5 mL) to give a dark green solution under nitrogen atmosphere and 0°C. Methoxymethylchloride (MOM-Cl, 0.3 g, 3.5 eq) was added, and a color change to a clear brown solution noted. N,N-Diisopropylethylamine (DIPEA, 0.48 g, 3.5 eq) was added dropwise with evolution of smoke, whereupon each new drop was added when the smoke had settled and disappeared. The reaction was monitored with TLC (Hex:EtOAc 1:2 + 2 drops TEA, R_f = 0.7), and terminated upon complete transformation of the aldehyde. Solvent and excess MOM-Cl was removed under vacuo to give a yellow oil. The product was dissolved in EtOAc and partitioned with distilled H₂O. The aqueous layer was extracted with EtOAc (x 4), and the combined organic fractions washed with cold 0.5 N HCl (x 2), 2 N NaOH (x 3) and brine (x 2) and dried with anhydrous MgSO₄. The solvent was removed under vacuo and the product purified with flash chromatography (Hex:EtOAc 1:2) to give 4-methoxy-2,3-bis(methoxymethoxy)benzaldehyde as a golden yellow/brown oil.

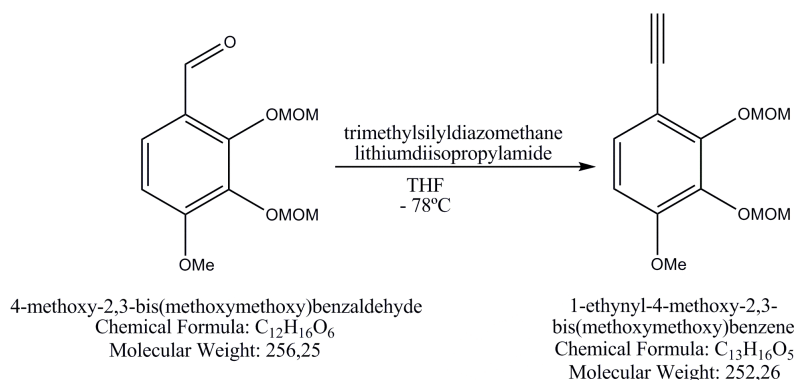
4-Methoxy-2,3-bis(methoxymethoxy)benzaldehyde; golden yellow/brown oil, 62-87% yield,

¹H NMR (300 MHz, CDCl₃) δ 10.25 (s, *J* = 3.7, 1H), 7.61 (d, 1H), 6.78 (d, *J* = 8.8, 1H), 5.24 (s, *J* = 3.5, 2H), 5.10 (s, 2H), 3.89 (s, 3H), 3.58 (s, *J* = 3.3, 3H), 3.52 (s, *J* = 4.2, 3H).

TLC: R_f = 0.6 Hex:EtOAc (1:2 + 2 ggt TEA).

4.8. 1-Ethynyl- 2,3-bis(MOM)-4-methoxybenzene

[11]



Trimethylsilyldiazomethane (2.0 M in hexane, 18 mmol, 1.25 eq) was added dropwise to a solution of lithium diisopropylamide (1.8 M in heptane/THF/ethylbenzene, 19 mmol, 1.38 eq) to give a yellow solution at -78°C and nitrogen atmosphere, and stirred for 1 h. A solution of 4-methoxy-2,3-bis(MOM)benzaldehyde (4 mmol, 1.026 g) in dry THF (9 mL) was subsequently added dropwise to give a clear orange solution, and stirred at -78°C for 1 h, then at room temperature for 2 h. The reaction was quenched with brine (5 mL), and extracted with EtOAc (8 mL x 4). The combined organic layers were washed with brine (8 mL) and water (8 mL), and dried with anhydrous MgSO₄. Solvent was removed in vacuo to give a golden/brown oil and the product purified with flash chromatography (Hex:EtOAc 1:2) to give 1-Ethynyl- 2,3-bis(methoxymethoxy)-4-methoxybenzene as an orange/brown oil in X % yield.

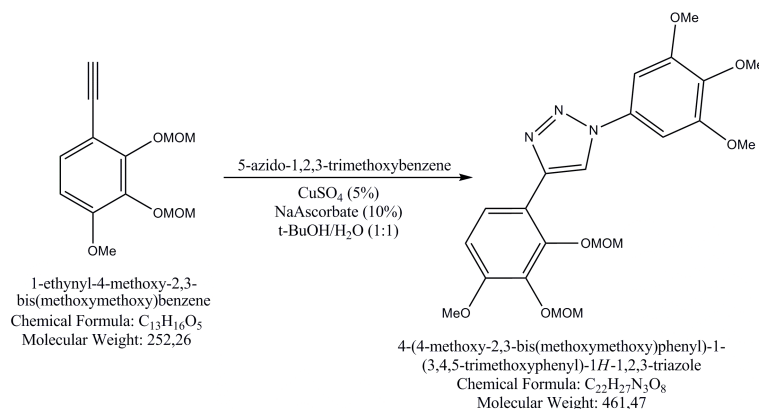
1-Ethynyl- 2,3-bis(methoxymethoxy)-4-methoxybenzene; orange/brown oil, 9.6-58% yield,

¹H NMR (300 MHz, CDCl₃) δ 7.19 (d, *J* = 8.7, 1H), 6.63 (d, *J* = 8.7, 1H), 5.25 (s, 2H), 5.10 (s, 2H), 3.83 (s, 4H), 3.62 (s, 3H), 3.57 (s, 3H), 3.17 (s, 1H).

TLC: R_f = 0.83 Hex: EtOAc (1:2)

4.9. 4-(4-Methoxy-2,3-bis(MOM)phenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole

[12]



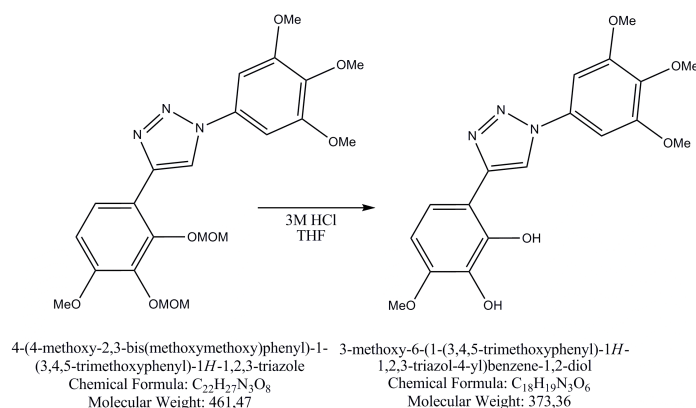
5-Azido-1,2,3-trimethoxybenzene (1 mmol, 0.209 g) and 1-ethynyl-4-methoxy-2,3-bis(methoxymethoxy)benzene (1 mmol, 0.257 g) was suspended in 5 mL *tert*-butanol / H_2O (1:1, 5 mL) to give a yellow solution. Addition of sodium ascorbate (10 mol %, 19.8 mg) and cuppersulfate pentahydrate (5 mol %, 13 mg) gave the solution a turbid appearance. The mixture was stirred at ambient temperature and monitored with TLC (Hex: EtOAc 1:1.5). Upon no further conversion, or when reaction was run to completion, the mixture was diluted with 15 mL cold H_2O and 4 mL 10% aqueous ammoniumbuffer (pH= 7) and stirred for additional 5 minutes. The precipitate was collected with a Büchner-filter and dried under suction to give 4-(4-methoxy-2,3-bis(methoxymethoxy)phenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole as a pale brown powder without further need of purification.

4-(4-Methoxy-2,3-bis(methoxymethoxy)phenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole; pale brown powder, 79- 81% yield,

1H NMR (300 MHz, $CDCl_3$) δ 8.53 (s, $J = 3.8$, 1H), 8.00 (d, $J = 3.6$, 8.8, 1H), 6.99 (s, 2H), 6.83 (d, $J = 3.9$, 8.9, 1H), 5.23 (s, 2H), 5.14 (s, 2H), 3.93 (s, 6H), 3.89 (s, 3H), 3.87 (s, 3H), 3.61 (s, $J = 3.1$, 3H), 3.41 (s, $J = 2.7$, 3H).

TLC: $R_f = 0.63$ Hex: EtOAc (1:1.5)

4.10.3-Methoxy-6-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-4-yl)benzene-1,2-diol



The triazole 4-(4-methoxy-2,3-bis(methoxymethoxy)phenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (0.2 mmol, 0.09 g) was dissolved in 6.5 mL 3 M HCL:THF (1:1) to give a clear brown/yellow solution and stirred at ambient temperature for 3 hours. The mixture was diluted with 10 mL H₂O and extracted with dichloromethane (5 mL x 3). The combined organic fractions were dried with anhydrous MgSO₄, and solvent removed in vacuo to give a golden brown oil. The product was purified with flash chromatography and deactivated silica₆₀ (deactivated with Hex: Silica₆₀: TEA, 1:1:1) using Hex:EtOAc (1:2) as eluent to afford 3-methoxy-6-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-4-yl)benzene-1,2-diol as a tan powder in 45 % yield.

For future reference it may be necessary to add 1% acetic acid to the eluent to prevent oxidation of the catechols.

3-Methoxy-6-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-4-yl)benzene-1,2-diol: tan powder, 45 % yield.

¹H NMR (300 MHz, CDCl₃) δ 10.25 (s, 1H), 8.14 (s, 1H), 7.10 (d, *J* = 8.7, 1H), 6.97 (s, 2H), 6.53 (d, *J* = 8.7, 1H), 5.52 (s, 2H), 3.93 (s, 6H), 3.90 (s, 3H), 3.88 (s, 3H).

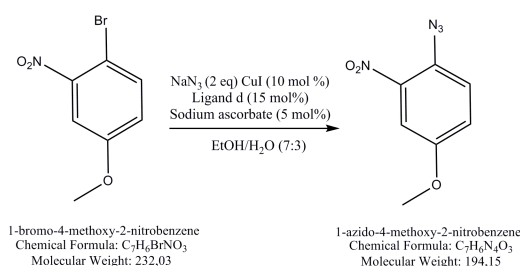
TLC: R_f = 0.43 Hex:EtOAc (1:2)

4.11. Synthesis of arylhalide to arylazide

A general procedure for the preparation of arylazide from the corresponding arylbromide was as follows:

Aryl bromide (2 mmol), sodium azide (4 mmol), sodium ascorbate (0.1 mmol), copper iodide (0.2 mmol), (1*S*,2*S*)-*N*1,*N*2-dimethyl-cyclohexane-1,2-diamine as ligand **d** (0.3 mmol), and 4 mL EtOH—H₂O (7:3) were introduced to a two-necked round-bottom flask equipped with a stirring bar and reflux condenser. The reaction was stirred under reflux and in an anaerobe atmosphere, and progression monitored with TLC. Upon reaction completion, or no further conversion noted, the mixture was cooled down to ambient temperature and the crude mixture purified either by extraction with EtOAc, and/or flash chromatography where necessary (Hex: EtOAc, 2:1) to yield the desired aryl azide.[22]

4.12. 1-Azido-4-methoxy-2-nitrobenzene



1-Bromo-4-methoxy-2-nitrobenzene (0.5 mmol, 0.116 g) and sodium azide (1 mmol, 65 mg, 2 eq) were solved in 0.5 mL EtOH-H₂O (7:3), and a solution of copper iodide (9.5 mg, 10 mol %) sodium ascorbate (5 mg, 5 mol %) (1*S*,2*S*)-*N*1,*N*2-dimethyl-cyclohexane-1,2-diamine (11 mg, 15 mol%) in 0.5 mL EtOH-H₂O (7:3) was added. The mixture was left to stir under reflux and nitrogen atmosphere, and monitored with TLC (Hex:EtOAc 2:1, R_f = 0.45). 10 mL H₂O was added and the mixture allowed to reach ambient temperature. The mixture was extracted with EtOAc (5 ml x 4), and the combined organic fractions dried with anhydrous MgSO₄. The solvent was removed under vacuo and the product purified with flash chromatography (Hex:EtOAc 2:1) to afford 1-azido-4-methoxy-2-nitrobenzene as a dark red oil.

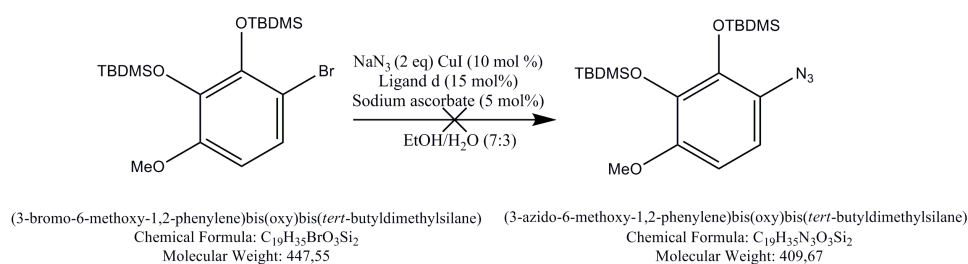
1-Azido-4-methoxy-2-nitrobenzene; red oil, 8-65% yield,

¹H NMR (300 MHz, CDCl₃) δ 7.53 (d, J = 2.9, 1H), 7.05 (dd, J = 3.0, 9.1, 1H), 6.74 (d, J = 9.1, 1H), 3.78 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 151.18, 140.30, 127.12, 120.51, 106.63, 56.24, 1.41.

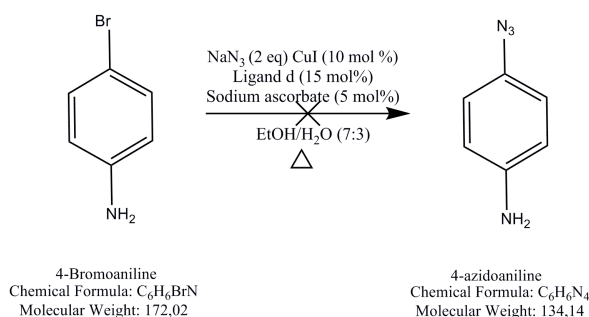
TLC: R_f = 0.43 Hex: EtOAc (2:1). IR: 2254 cm⁻¹

4.13. 1-Azido-2,3bis(TBDMS)-4-methoxybenzene



Sodium ascorbate (5 mol %, 0.5 mg), (1*S*,2*S*)-*N*1,*N*2-dimethyl-cyclohexane-1,2-diamine (15 mol %, 11 mg), copper iodide (10 mol %, 9.5 mg), 1-bromo-2,3 bis(*tert*butyldimethylsilyloxy)-4-methoxybenzene (0.5 mmol, 0.12 g) and sodium azide (2 eq, 6.5 mg) were successively solved in 0.5 mL EtOH-H₂O (7:3), to give a blue frothing solution. The mixture was stirred under reflux and nitrogen atmosphere for 1 h, and monitored with TLC (Hex: EtOAc 2:1). The solution was added 5 mL H₂O and allowed to cool down to room temperature before extraction with EtOAc. The combined organic fractions were dried with anhydrous MgSO₄ and the solvent removed under vacuo to give a motley white and dark green/brown waxy solid.

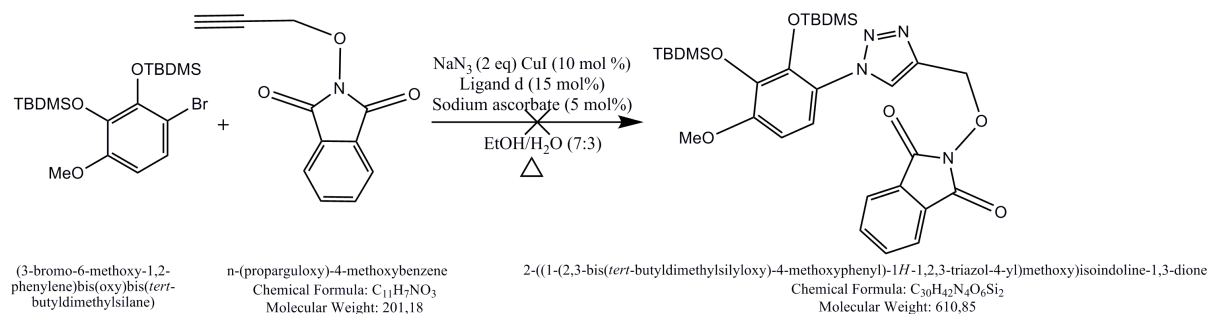
4.14. 4-Azidoaniline



A two-necked round-bottom flask equipped with reflux-condenser and a stirring bar was arranged and 4-bromoaniline (0.5 mmol, 86 mg) and sodium azide (1 mmol, 65 mg, 2 eq) were solved in 0,5 mL EtOH-H₂O (7:3) under nitrogen atmosphere. A solution of copper iodide (9.5 mg, 10 mol %) sodium ascorbate (5 mg, 5 mol %), (1*S*,2*S*)-*N*1,*N*2-dimethyl-cyclohexane-1,2-diamine (11 mg, 15 mol%) in 0,5 mL EtOH-H₂O (7:3) were introduced and a change in color to blue/green noted. The mixture was stirred under reflux and nitrogen atmosphere, and monitored with TLC (Hex: EtOAc, 2:1). No trace of product was noticeable on the TLC-sheet and the reaction discarded.

4.15. 2-((1-(2,3-Bis(TBDMS)-4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)isoindoline-1,3-dione

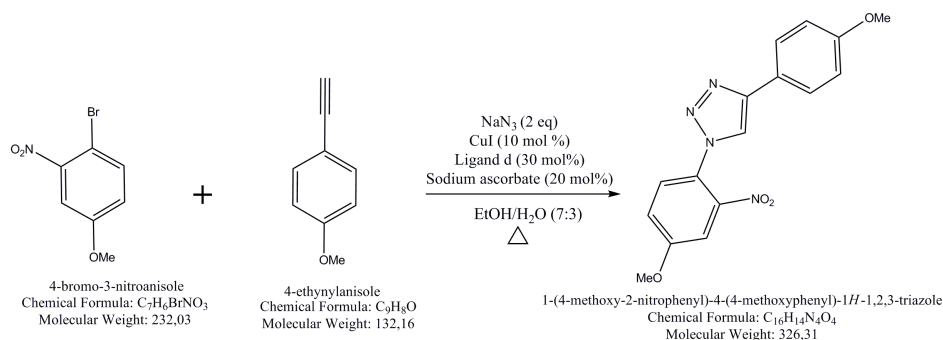
[12, 22]



A two-necked round-bottom flask with reflux-condenser and stirring bar was arranged, followed by introduction of arylbromide 1-bromo-2,3 bis(*tert*-butyldimethylsilyloxy)-4-methoxybenzene (0.5 mmol, 0.223g), the alkyne *n*-(proparguloxo)-phthalimide (0.5 mmol, 0.10g, 1 eq) and sodium azide (1 mmol, 0.065g, 2 eq). 1.5 mL *tert*-ButOH/ H_2O (1:1) was added. The solution was left to stir for 24h before dissolving the arylbromide. Successively were added sodium ascorbate (0.01g, 10 mol %), ligand (1*S*,2*S*)-*N*1,*N*2-dimethylcyclohexane-1,2-diamine (0.011g, 15 mol %) and copper iodide (9.5 mg, 10 mol %). The solution was stirred under reflux, upon addition of new portions of sodium ascorbate, ligand and copper iodide after 6 hours. The reaction was monitored with TLC (Hex:EtOAc, 1:2). Upon no further conversion according to TLC the mixture was allowed to cool down to ambient temperature. The mixture was diluted with 25 mL cold H_2O , and added 10 mL 10% aqueous ammonia (pH~8.5), and stirred for a minimum of 5-10 minutes. The precipitate was collected with a Büchner-filter and dried under suction. No product was achieved according to TLC of precipitate, and the reaction discarded.

4.16. 1-(4-Methoxy-2-nitrophenyl)-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole

[12, 22]



A two-necked round-bottom flask with reflux-condenser and stirring bar was arranged and arylbromide 4-Bromo-3-nitroanisole (0.5 mmol, 66.1 mg), 1-Ethynyl-anisole (0.5 mmol, 0.166g, 1 eq) and sodium azide (4 mmol, 0.065g, 4 eq) with 2 mL EtOH/H₂O (7:3) was introduced. Successive addition of sodium ascorbate (19 mg, 20 mol %), ligand (1*S*,2*S*)-*N*1,*N*2-dimethyl-cyclohexane-1,2-diamine (21.3 mg, 30 mol %) and copper iodide (20 mg, 20 mol %) was followed. The solution was stirred under reflux and monitored with TLC (Hex/EtOAc 4:1). A color change from mossy green to brownish red was noted. Upon no further conversion according to TLC was shown, the mixture was allowed to cool down to ambient temperature. The solution was diluted with 25 mL cold H₂O, and added 10 mL 10% aqueous ammonia (pH~8,5), before left to stir for a minimum of 5-10 minutes. The precipitate was collected with a Büchner-filter and dried under suction. The precipitate was resolved in dichloromethane to separate the product from the filter, and dried with anhydrous MgSO₄ to remove traces of water. Removal of the solvent under vacuo afforded 1-(4-methoxy-2-nitrophenyl)-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole as a beige powder.

1-(4-Methoxy-2-nitrophenyl)-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole;

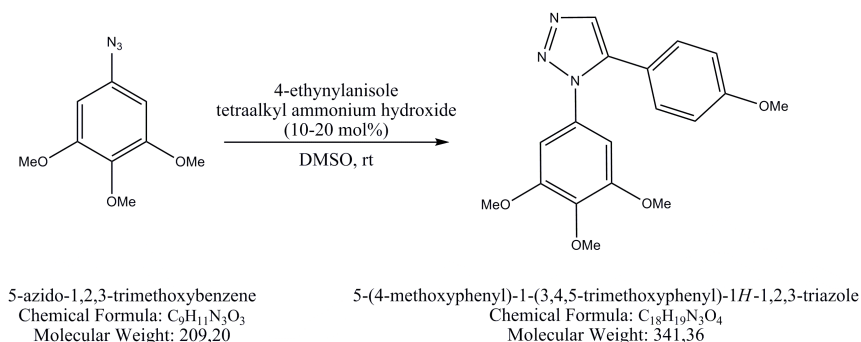
beige powder , 7 % yield, meltingpoint: not obtainable due low yield,

¹H NMR (300 MHz, CDCl₃) δ 7.91 (s, 1H), 7.81 (d, *J* = 8.8, 2H), 7.58 – 7.55 (m, 1H), 7.53 (s, 1H), 7.25 (s, 2H), 6.98 (d, *J* = 8.8, 2H), 3.95 (s, 3H), 3.85 (s, 3H)

TLC: R_f = 0.28 (Hex/EtOAc 4:1)

Synthesis of 5-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole by

4.17. *Tetra*-alkyl ammonium hydroxide analogues; test of reactivity



To a solution of 5-azido-1,2,3-trimethoxybenzene (1 eq, 0.5 mmol, 0.101 g) and 4-ethynylanisole (1 eq, 0.5 mmol, 0.066 g) in 3 mL DMSO, the *tetra*-alkyl ammonium hydroxide analogue was added and stirred at ambient temperature. The reaction was monitored with TLC (Hex: EtOAc 4:1) and H_2O (50 % v/v) added after 1-2 days and left to stir for 1 hour. If precipitation occurred it was collected with a Büchner filter and dried under suction to afford 5-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole as a beige powder.

Where precipitation was afforded in low yield the filter was washed with EtOAc, dried with $MgSO_4$ and solvent removed in vacuo. The result was analysed on NMR.

In case of *tetra*-butyl the mixture was decided extracted with EtOAc without attempt at filtrating an eventual precipitation. The organic fase was washed with brine and water before dried with anhydrous $MgSO_4$ and the solvent removed in vacuo. The product was solved in 10 dropps of EtOAc and partitioned with hexane followed by filtration of precipitation.

Additionally two parallel samples of *tetra*-ethyl ammonium hydroxide were dried on vacuum line for two days. One sample was resolved in MeOH (25 % solution) and the other used as dry substance.

Results from the different analogues were

<i>Tetra-methyl ammonium hydroxide</i> (25% in MeOH)			
20 mol %	0.04 g	< 2 %	Precipitate
1 equivalent	0.18 g	< 2 %	Precipitate
<i>Tetra-ethyl ammonium hydroxide</i> (25 % in H₂O)			
20 mol %	0.06 g	34 %	Precipitate
1 equivalent	0.3 g	60 %	Precipitate
1 equivalent dried	-	75 %	Precipitate
1 eq (25 % MeOH)	-	68 %	Precipitate
<i>Tetra-butyl ammonium hydroxide</i> (25% in MeOH)			
20 mol %	0.1 g	~ 6 %	Extracted
1 equivalent	0.5 g	-	Discharged

***Tetra-methyl ammonium hydroxide* (25% in MeOH)**

¹H NMR (300 MHz, CDCl₃) δ 7.82 (s, 3H), 6.77 – 6.63 (m, 11H), 5.07 – 4.95 (m, 11H), 3.82 (t, *J* = 5.3, 19H), 3.66 (s, 16H), 3.49 (s, 9H), 2.96 (d, *J* = 8.5, 8H), 2.58 (s, 3H), 2.00 (d, *J* = 8.1, 1H), 1.23 (t, *J* = 7.1, 2H), 0.04 (s, 1H).

***Tetra-ethyl ammonium hydroxide* (25 % in H₂O) afforded**

5-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole 33-68% yield,

¹H NMR (300 MHz, CDCl₃) δ 7.80 (s, 1H), 7.17 (d, *J* = 8.8, 2H), 6.87 (d, *J* = 8.8, 2H), 6.56 (s, 2H), 3.86 (s, 3H), 3.80 (s, 3H), 3.71 (s, 6H).

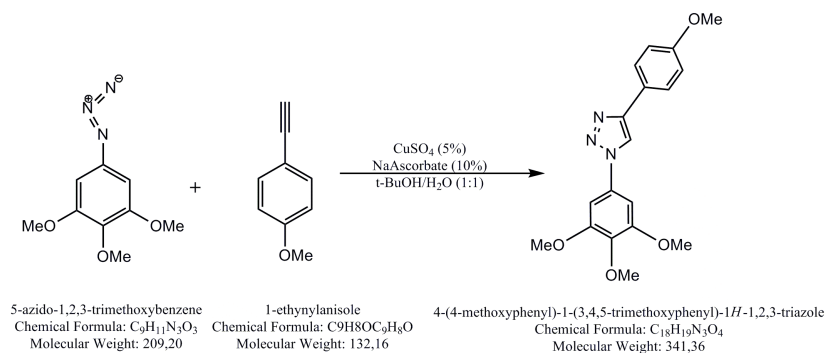
¹³C NMR (75 MHz, CDCl₃) δ 160.70, 153.87, 138.86, 130.36, 119.38, 114.69, 103.22, 77.85, 77.63, 77.42, 77.00, 61.43, 56.63, 55.76.

TLC: R_f = 0.28 (Hex: EtOAc 1:1)

***Tetra-butyl ammonium hydroxide* (25% in MeOH)**

¹H NMR (300 MHz, CDCl₃) δ 7.88 – 7.78 (m, 0H), 7.14 (s, 0H), 6.83 (d, *J* = 8.6, 1H), 6.61 (s, 1H), 6.29 (s, 2H), 3.88 (s, 1H), 3.85 (s, 7H), 3.81 (s, 4H), 3.73 (s, 1H), 1.27 (s, 2H).

4.18. 4-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole



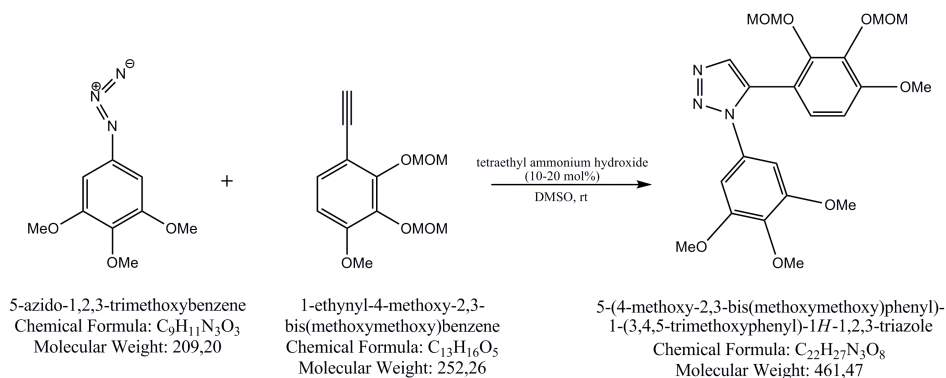
5-Azido-1,2,3-trimethoxybenzene (0.5 mmol, 0.105 g) and 1-ethynylanisole (0.5 mmol, 7 mg) was suspended in 3 mL *tert*-butanol / H₂O (1:1 mL) to give a yellow solution. Sodium ascorbate (10 mol %, 10 mg) and copper sulfate pentahydrate (5 mol %, ~1 mg) was added which gave the solution an instant turbid appearance. Additional 1 mL of *tert*-butanol / H₂O (1:1 mL) was added to dilute the thick solution. The mixture was stirred at ambient temperature for an hour and monitoring with TLC not attempted due to the thick consistency. The mixture was diluted with 15 mL cold H₂O and 4 mL 10% aqueous ammonium buffer (pH ≈ 7) and stirred for additional 5 minutes. The precipitate was collected with a Büchner-filter and dried under suction to give 4-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole as a pale brown powder without further need of purification.

4-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole:

pale brown powder, 70 % yield,

¹H NMR (300 MHz, CDCl₃) δ 8.04 (s, 1H), 7.81 (d, *J* = 8.8, 2H), 6.97 (t, *J* = 4.4, 4H), 3.93 (s, 6H), 3.88 (s, 3H), 3.84 (s, 3H).

4.19. 5-(4-Methoxy-2,3-bis(MOM)phenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole



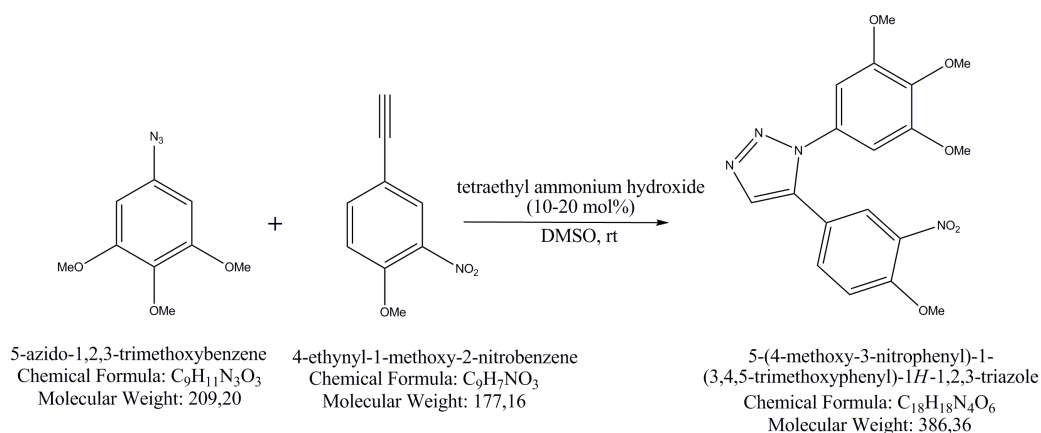
To a solution of 5-Azido-1,2,3-trimethoxybenzene (0.5 mmol, 0.101 g) and 1-ethynyl-4-methoxy-2,3-bis(methoxymethoxy)benzene (0.5 mmol, 0.126 g, 1 eq) in 2.7 mL DMSO was added *tetra*-ethyl- ammonium hydroxide (25 % in H₂O) (20 mol %, 0.06 g, 0.3 mL) and stirred at ambient temperature for a week. The reaction was not monitored with TLC. The reaction was quenched with 15 mL H₂O and stirred for a minimum of 5-10 minutes before collecting the precipitate with a Büchner-filter. The precipitate was dried under suction to afford 1-(4-methoxy-2-nitrophenyl)-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole in 3.5 % yield.

5-(4-Methoxy-2,3-bis(methoxymethoxy)phenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole: 3.5%, appearance and melting point unknown due to low yield,

¹H NMR (300 MHz, CDCl₃) δ 7.82 (s, 1H), 7.24 (s, 0H), 6.69 (dd, *J* = 3.0, 5.7, 3H), 5.07 – 4.95 (m, 4H), 3.85 (t, *J* = 5.3, 7H), 3.70 (s, 6H), 3.53 (s, 3H), 2.99 (d, *J* = 8.5, 3H), 2.62 (s, 1H), 2.05 (d, *J* = 8.1, 0H), 1.26 (t, *J* = 7.1, 1H), 0.07 (s, 1H)

R_f = not obtainable due to low yield.

4.20. 5-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole



To a solution of 5-Azido-1,2,3-trimethoxybenzene (0.5 mmol, 0.101 g) and 1-Ethynyl-1-methoxy-2-nitrobenzene (0.5 mmol, 0.089 g, 1 eq) in 2.7 mL DMSO, was added *tetra*-ethyl-ammonium hydroxide (25 % in H₂O) (20 mol %, 0.06 g, 0.3 mL) and stirred at ambient temperature. The reaction was monitored with TLC (Hex: EtOAc 1:1) and added H₂O (50 % v/v) when no further development was shown by TLC, and stirred for a minimum of 5-10 minutes before precipitate was attempted collected with a Büchner-filter. The solution did not contain a precipitate, and was extracted with EtOAc (20 mL*3) and the combined organic fractions washed with brine (100 mL) and water (100 mL*2). The solvent was dried with anhydrous MgSO₄ and removed in vacuo. The product was purified with flash chromatography (Hex: EtOAc 1:1) to afford 5-(4-methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole as a pale brown powder in 17 % yield.

5-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole: pale brown powder, 17 % yield,

¹H NMR (300 MHz, CDCl₃) δ 7.87 (s, 1H), 7.82 (d, *J* = 2.3, 1H), 7.35 (dd, *J* = 2.3, 8.8, 1H), 7.05 (d, *J* = 8.8, 1H), 6.55 (s, 2H), 3.97 (d, *J* = 7.7, 3H), 3.85 (d, *J* = 8.5, 3H), 3.73 (d, *J* = 9.7, 6H),

TLC: R_f = 0.43 (Hex: EtOAc 1:1)

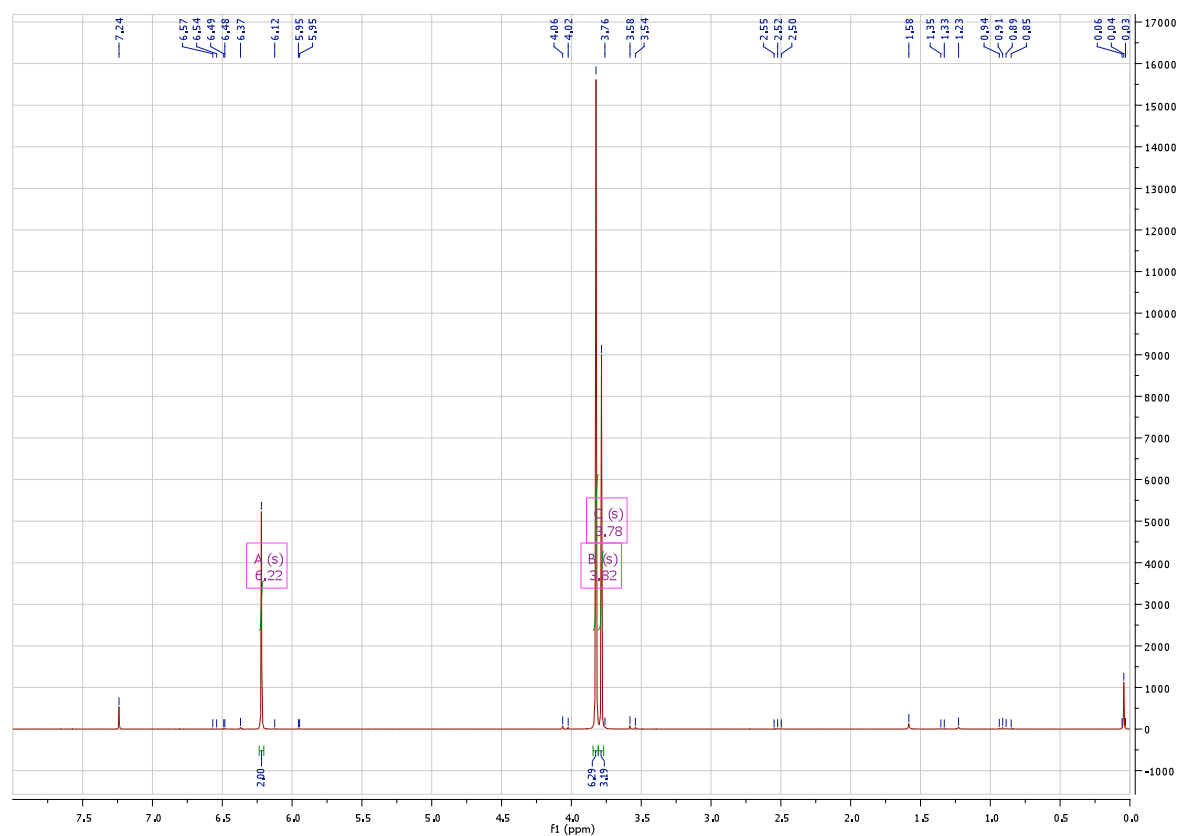
5. References

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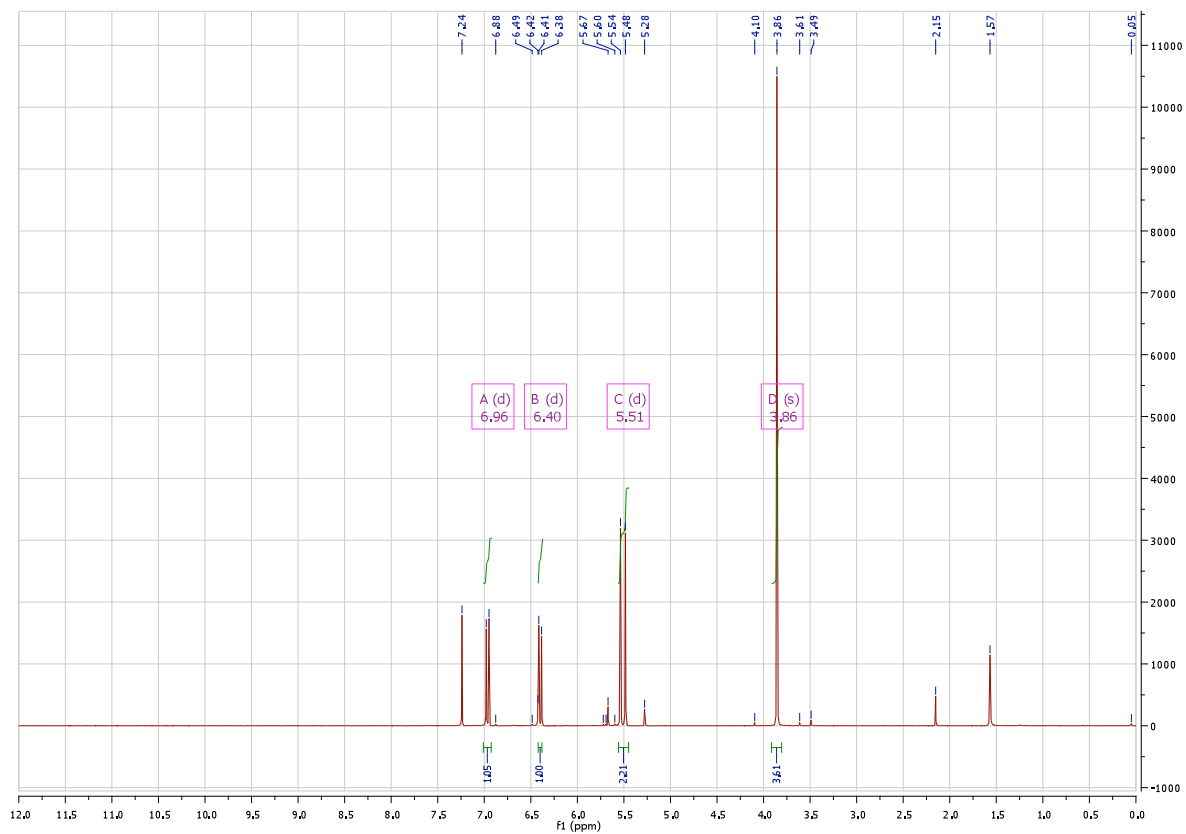
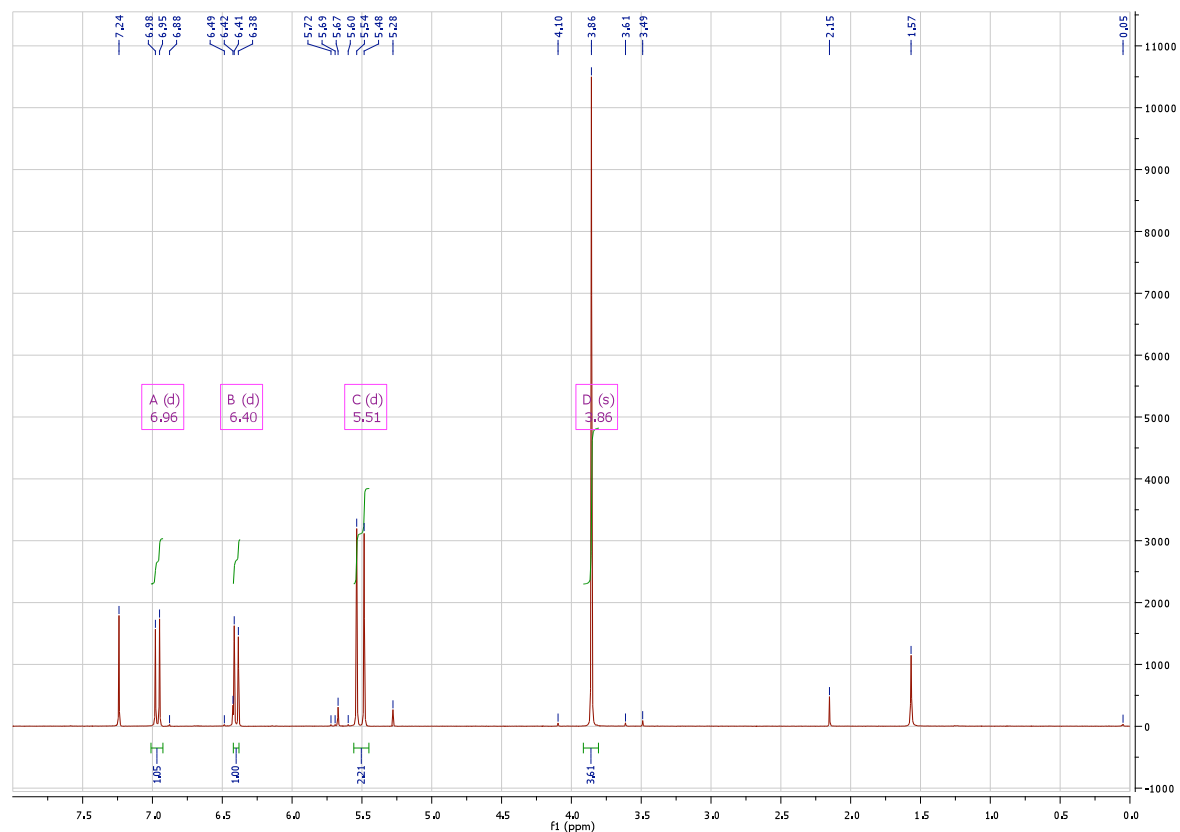
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6. Appendix:

6.1. 5-Azido-1,2,3-trimethoxybenzene

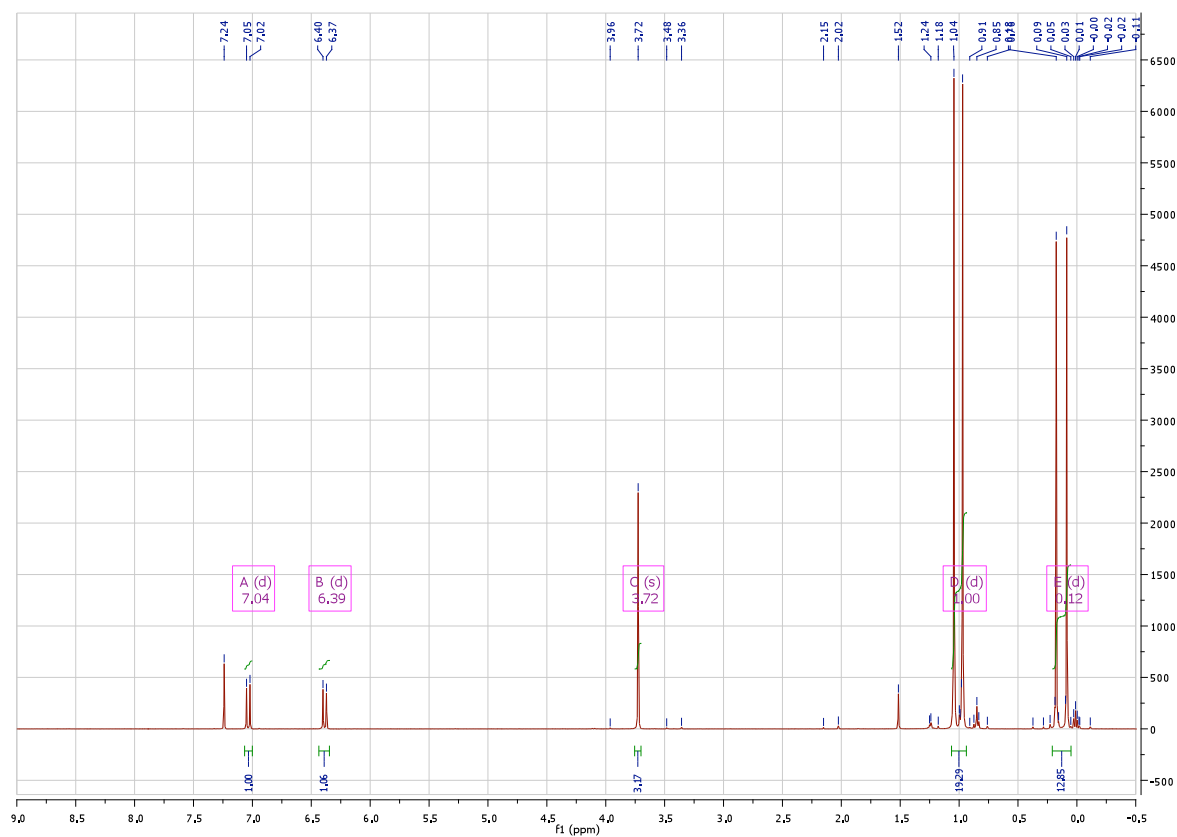


6.2. 1-Bromo-2, 3-dihydroxy-4-methoxybenzen

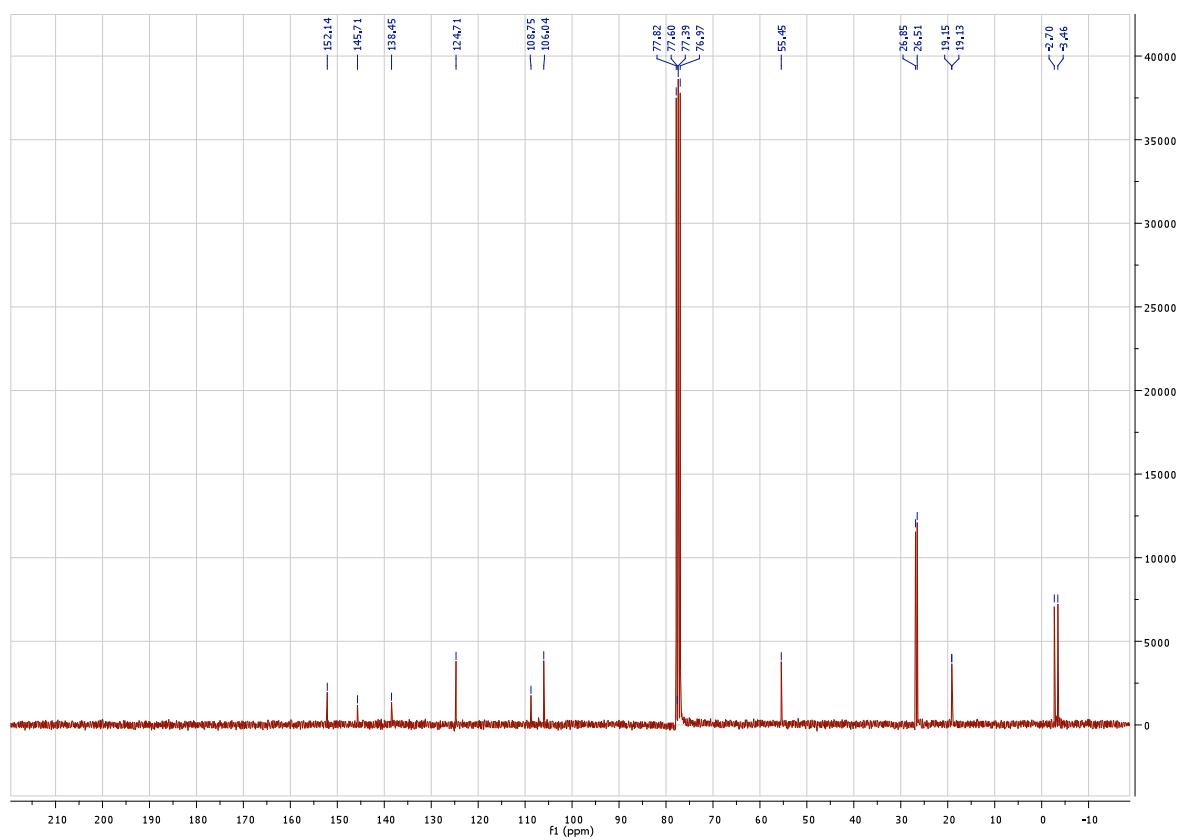


No aldehyde signal at 10-11 ppm confirms successful conversion

6.3. 1-Bromo-2,3bis (TBDMS)- 4-methoxybenzen

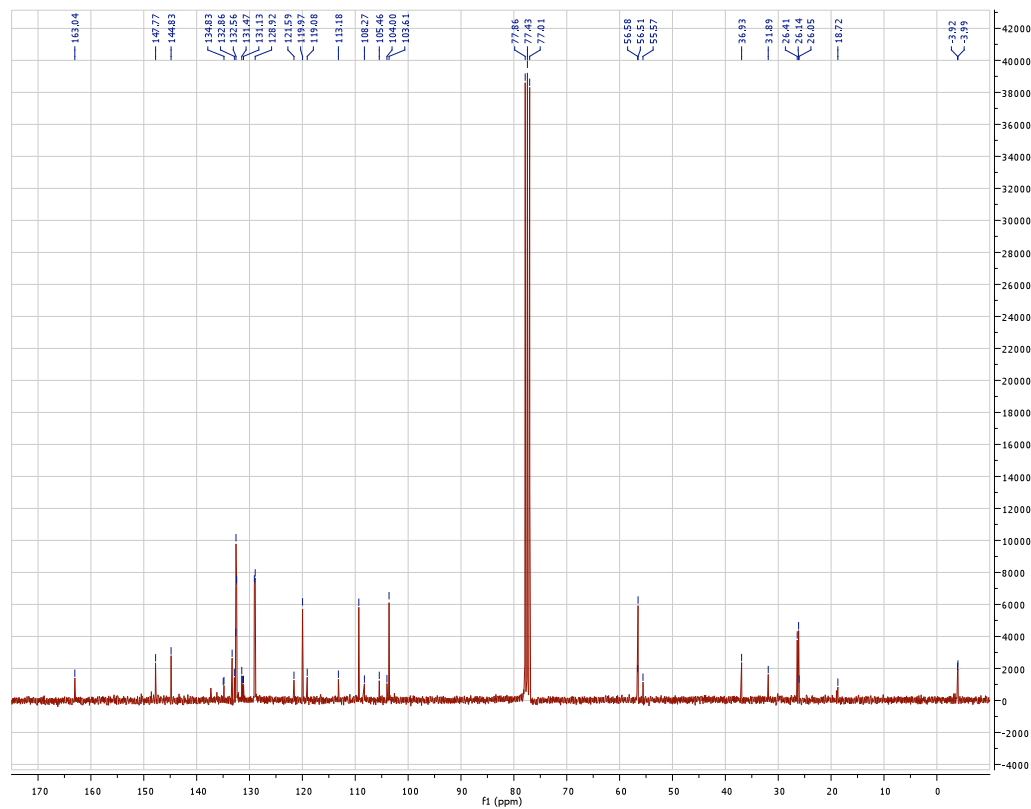
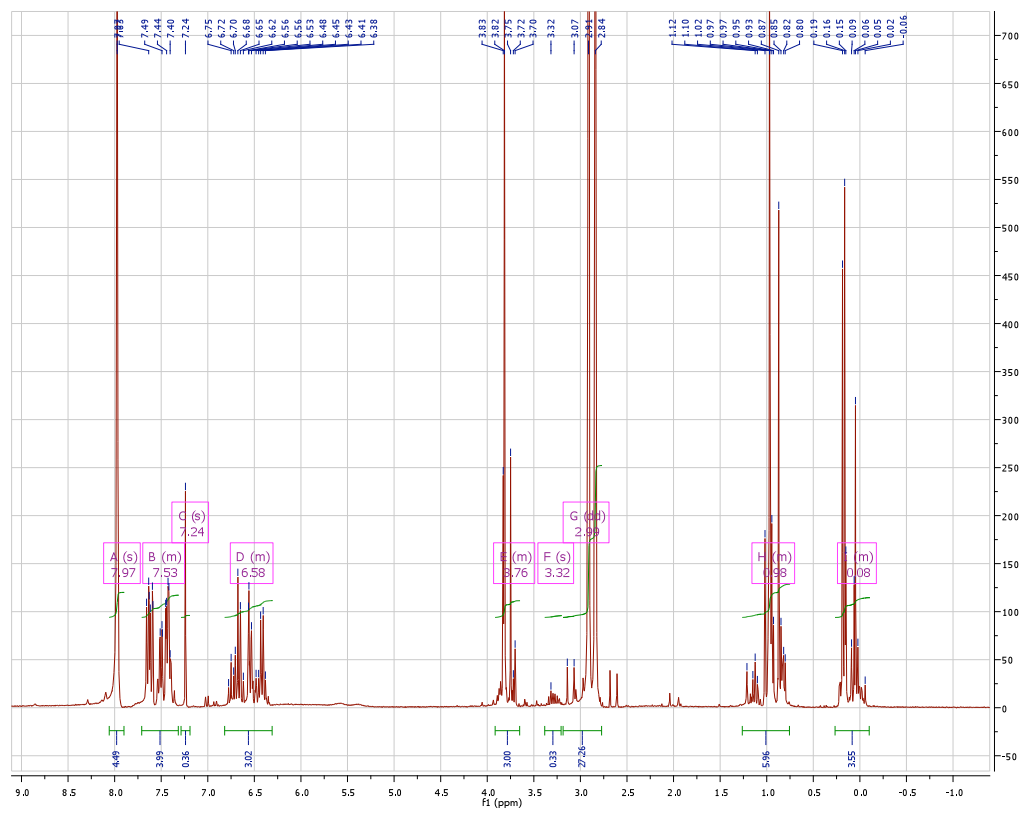


1-Bromo-2,3bis (TBDMS)- 4-methoxybenzen



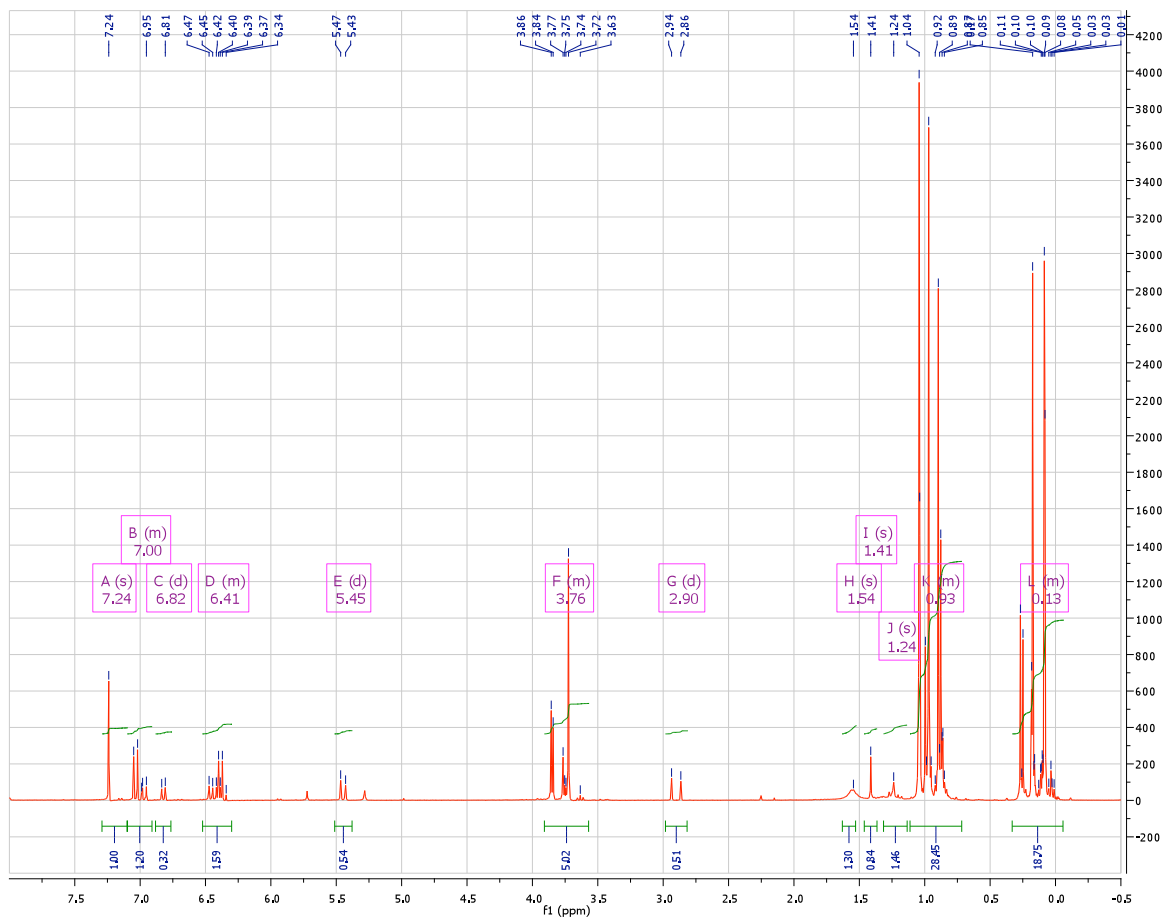
No alkyne signal in 70-80 ppm

6.4. 1-((Trimethylsilyl)ethynyl)-2,3bis(TBDMS)-4-methoxybenzene

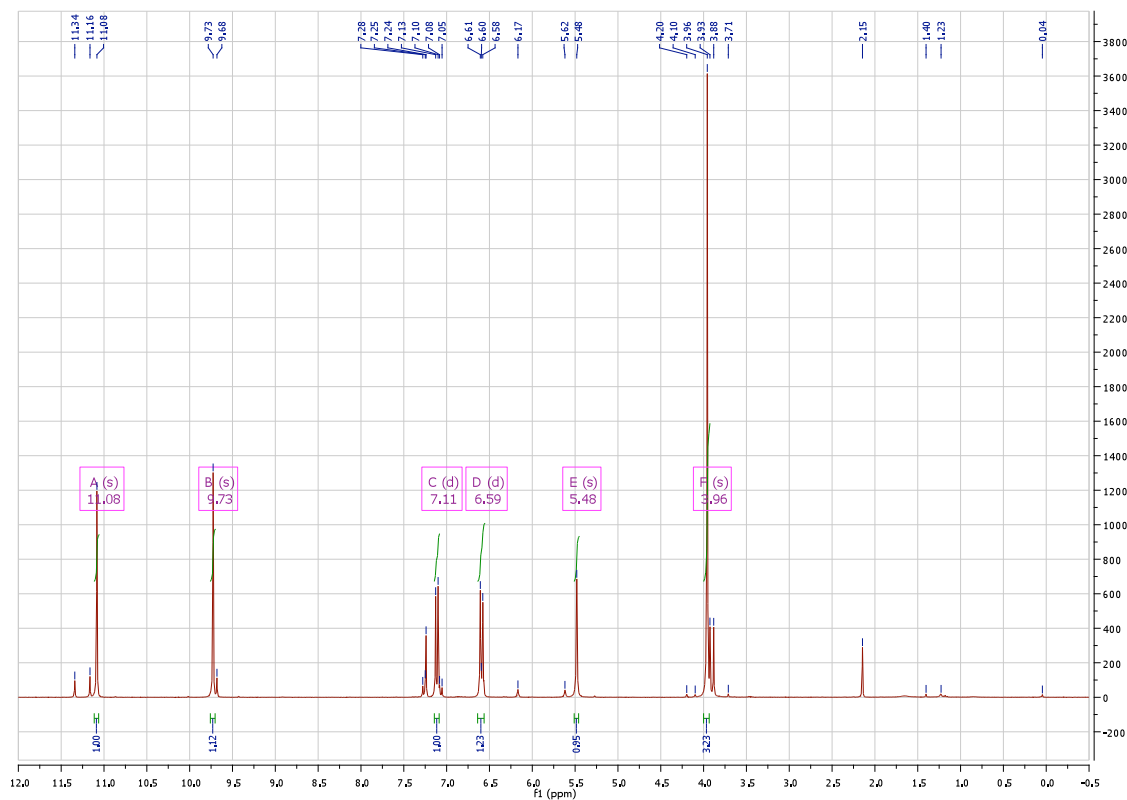


No alkyne signal in 70-80 ppm

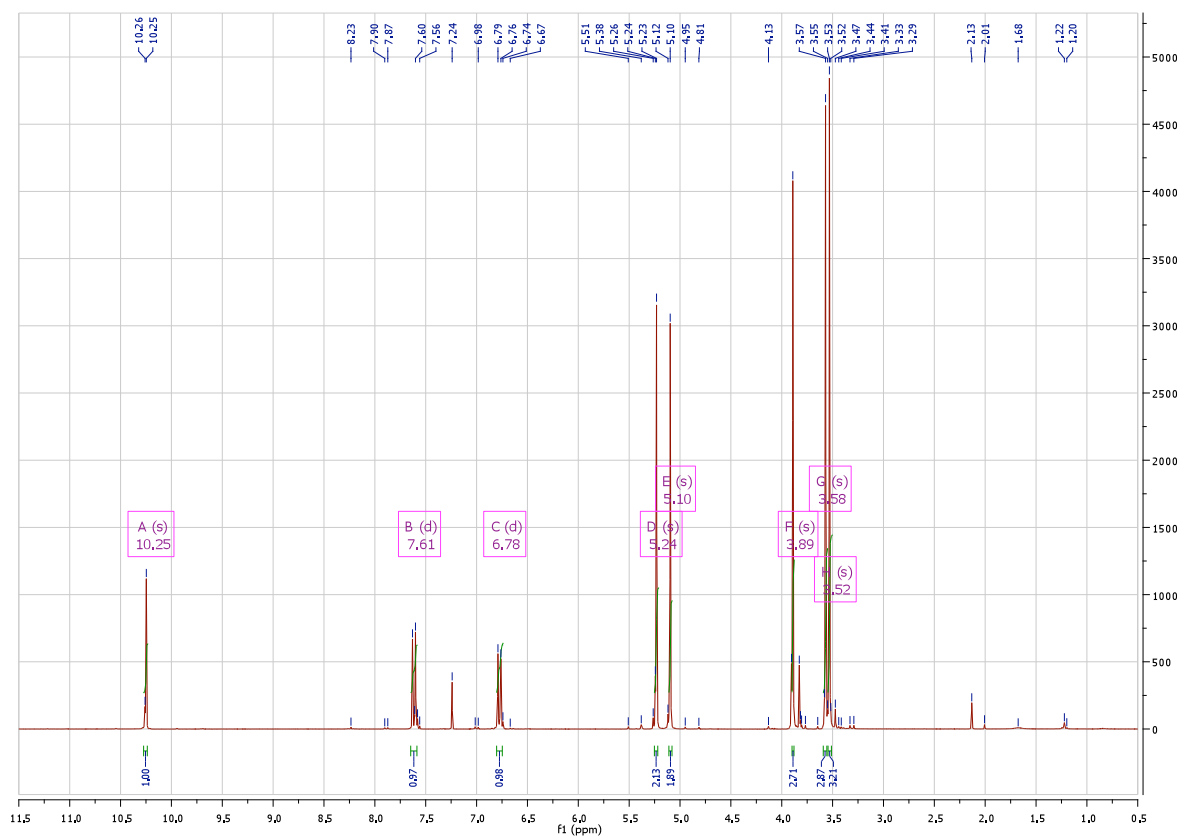
6.5. 2,3-Bis(TBDMS)-4-methoxybenzaldehyde



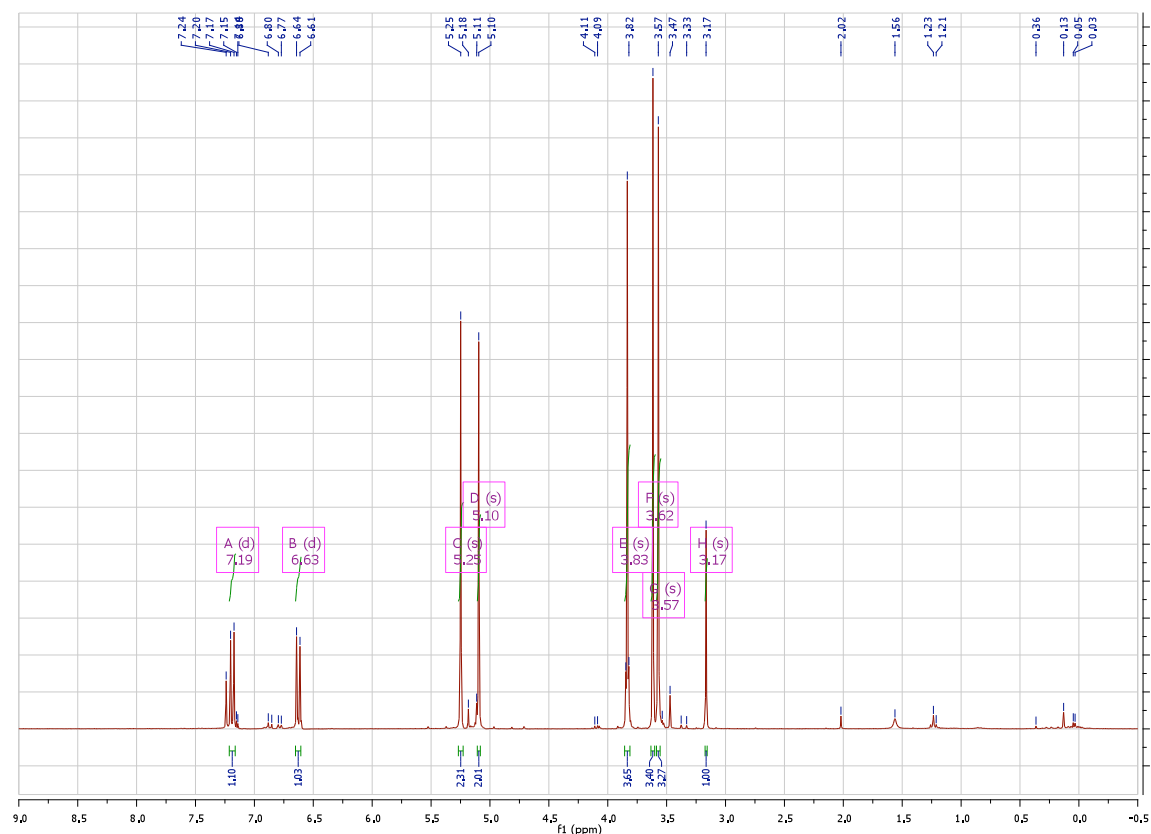
6.6. 2,3-Dihydroxy-4-methoxybenzaldehyde



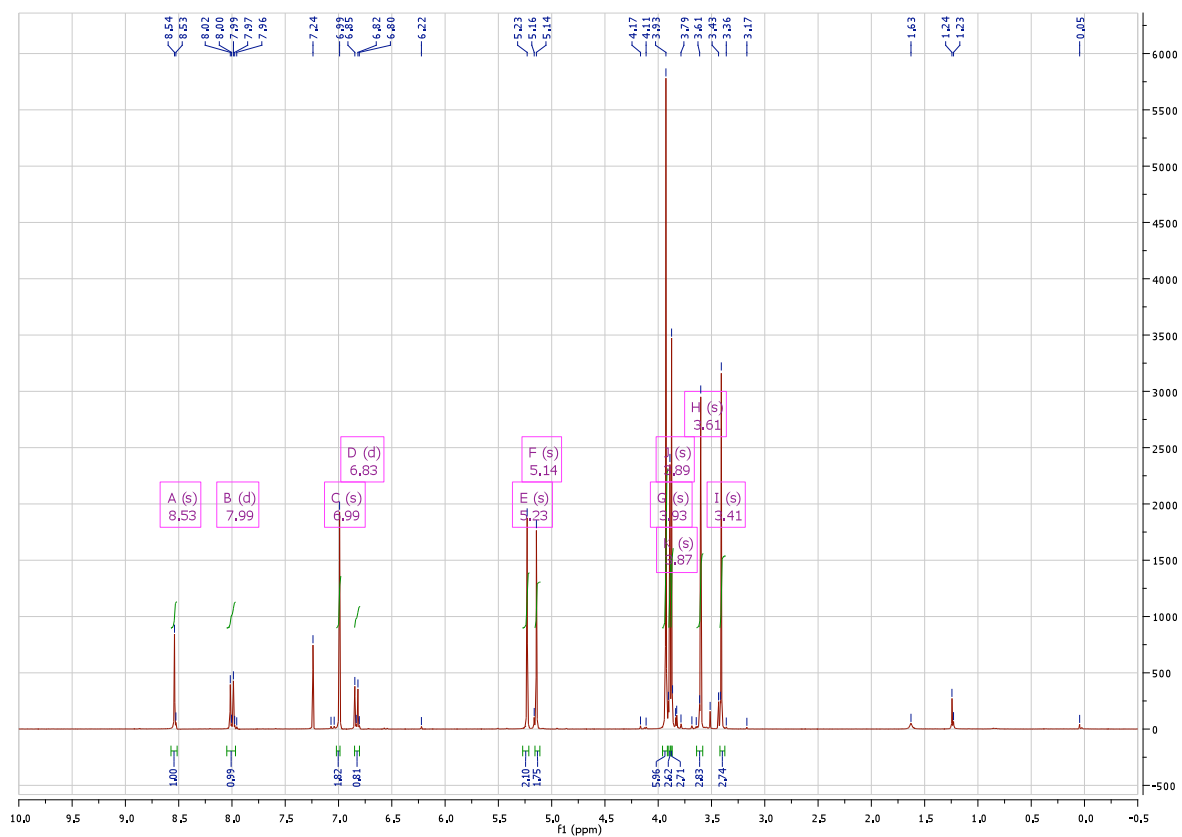
6.7. 4-Methoxy-2,3-bis(MOM)benzaldehyde



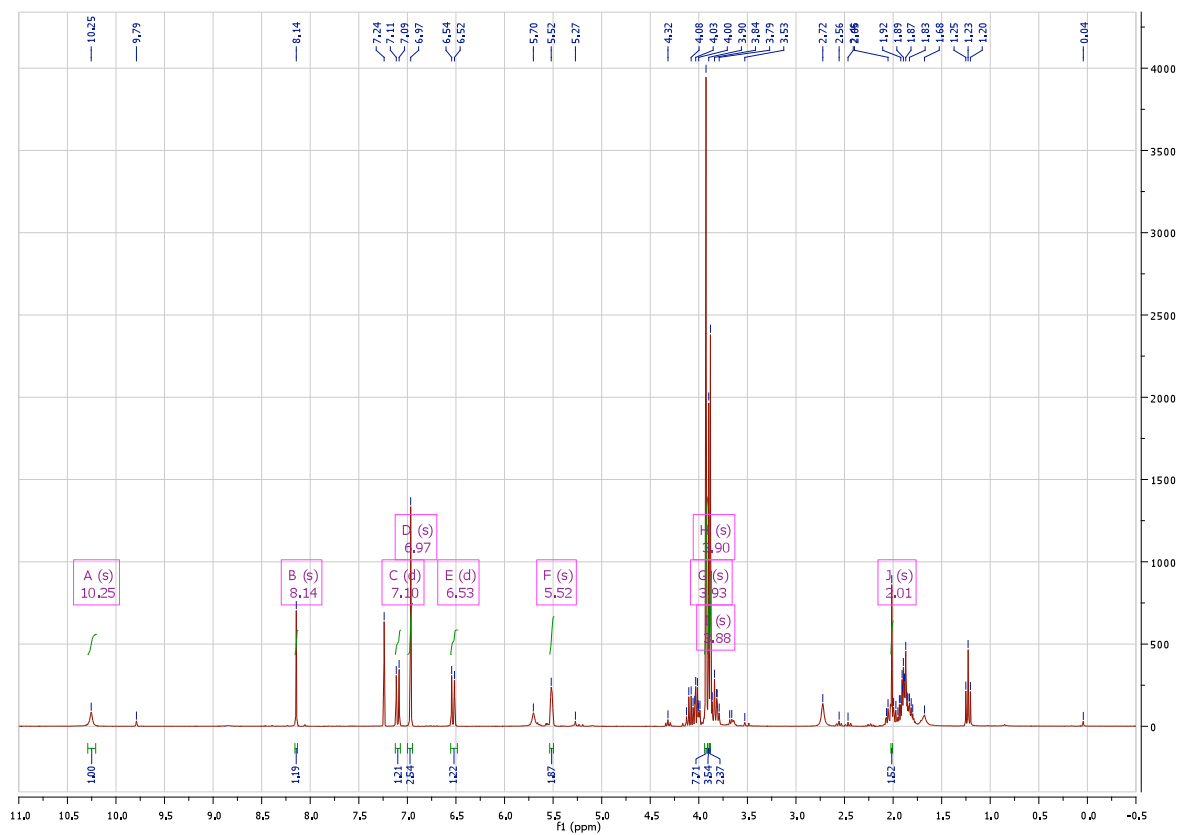
6.8. 1-Ethynyl-2,3-bis(MOM)-4-methoxybenzene



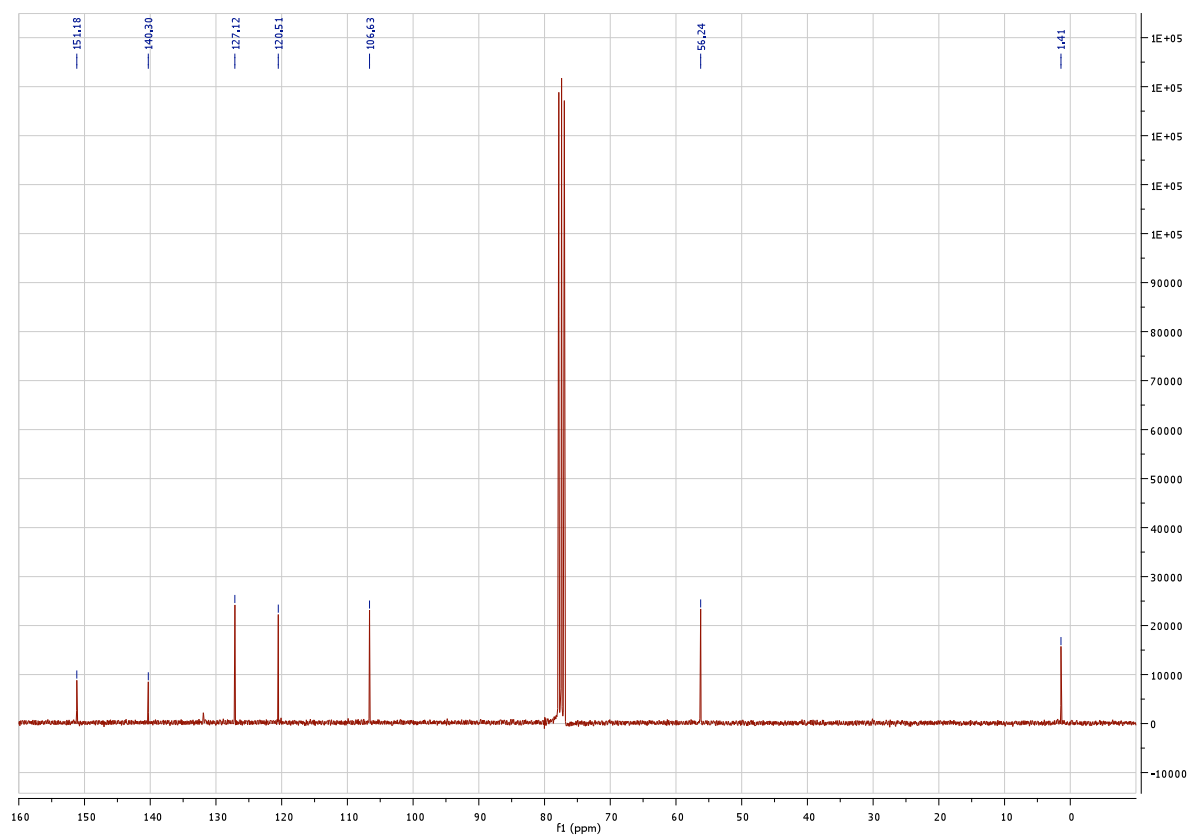
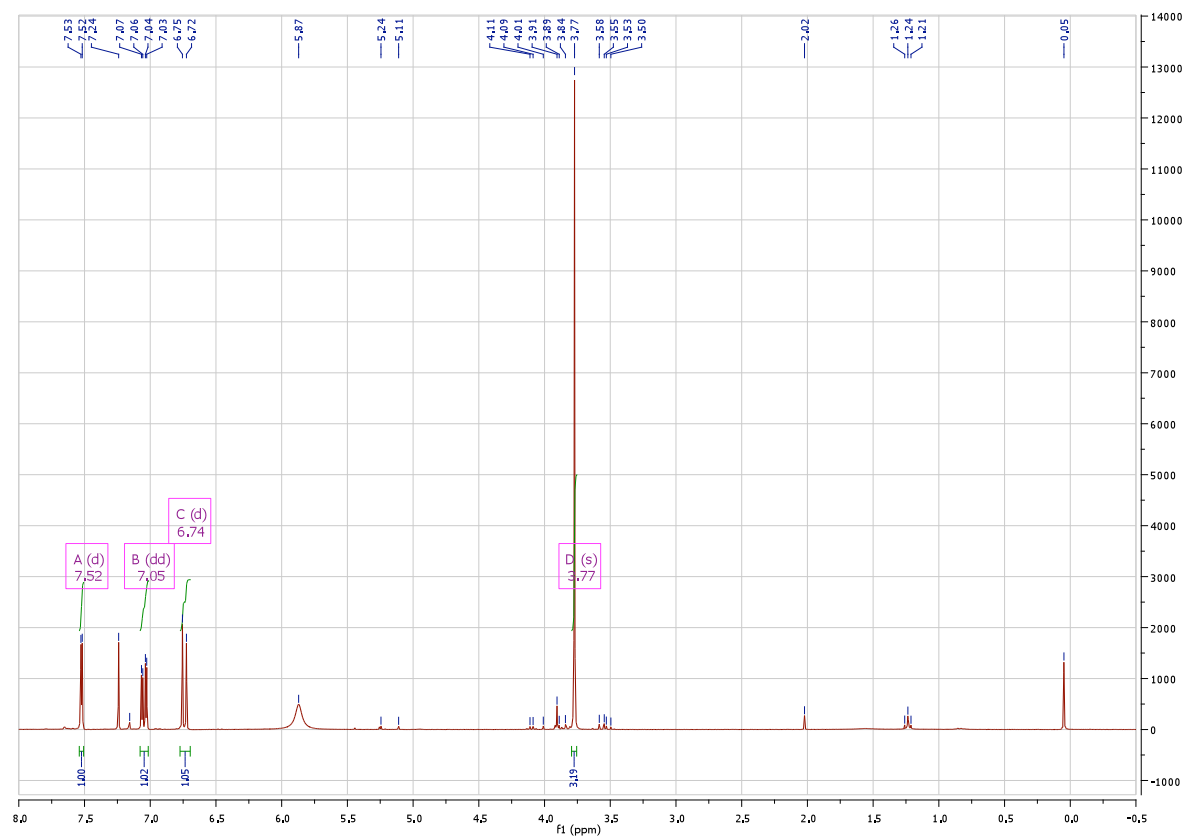
6.9. 4-(4-Methoxy-2,3-bis(MOM)phenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole



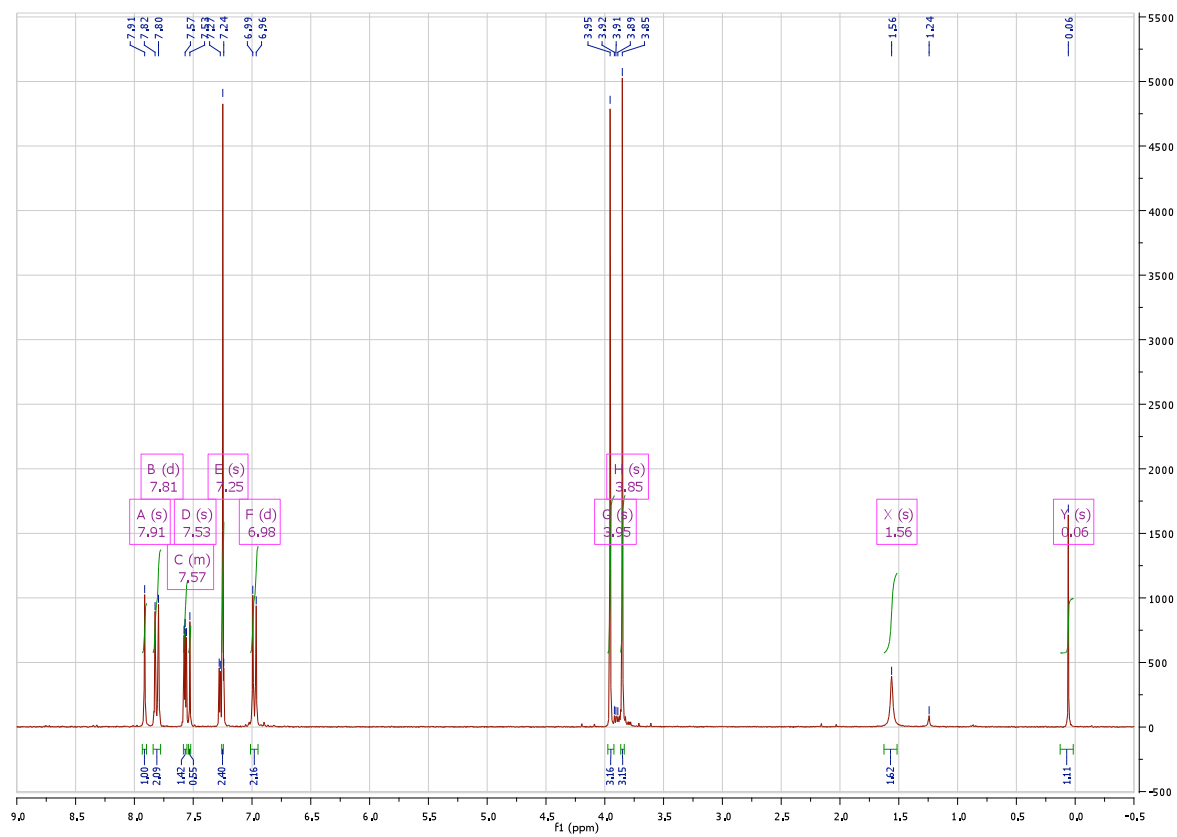
6.10. 3-Methoxy-6-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-4-yl)benzene-1,2-diol:



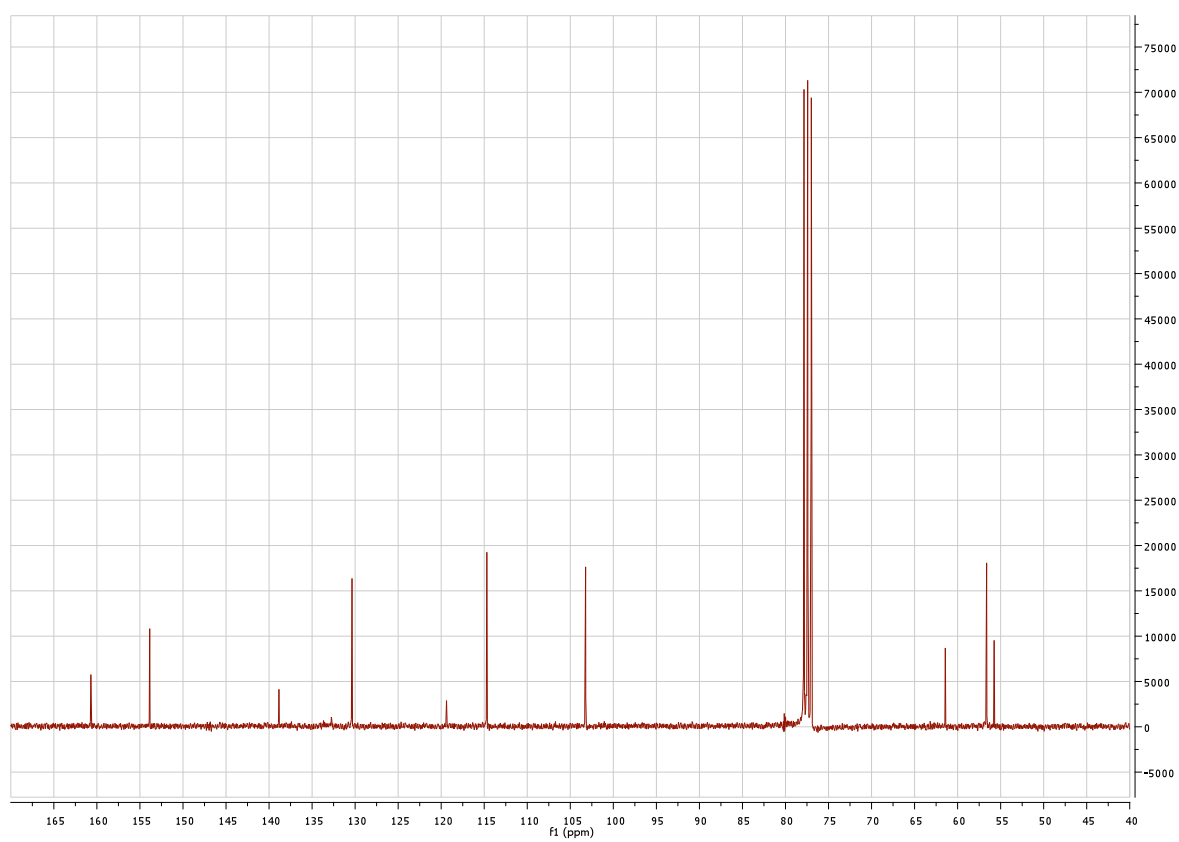
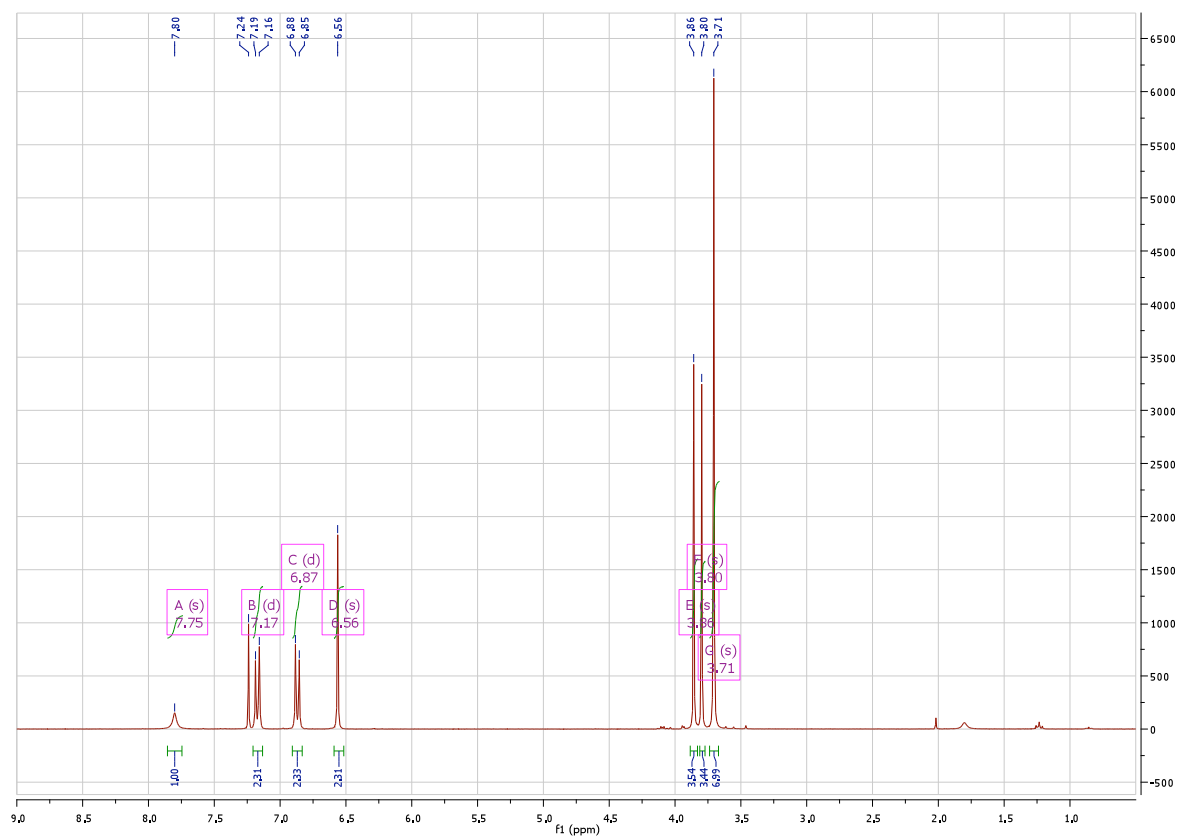
6.11.1-Azido-4-methoxy-2-nitrobenzene



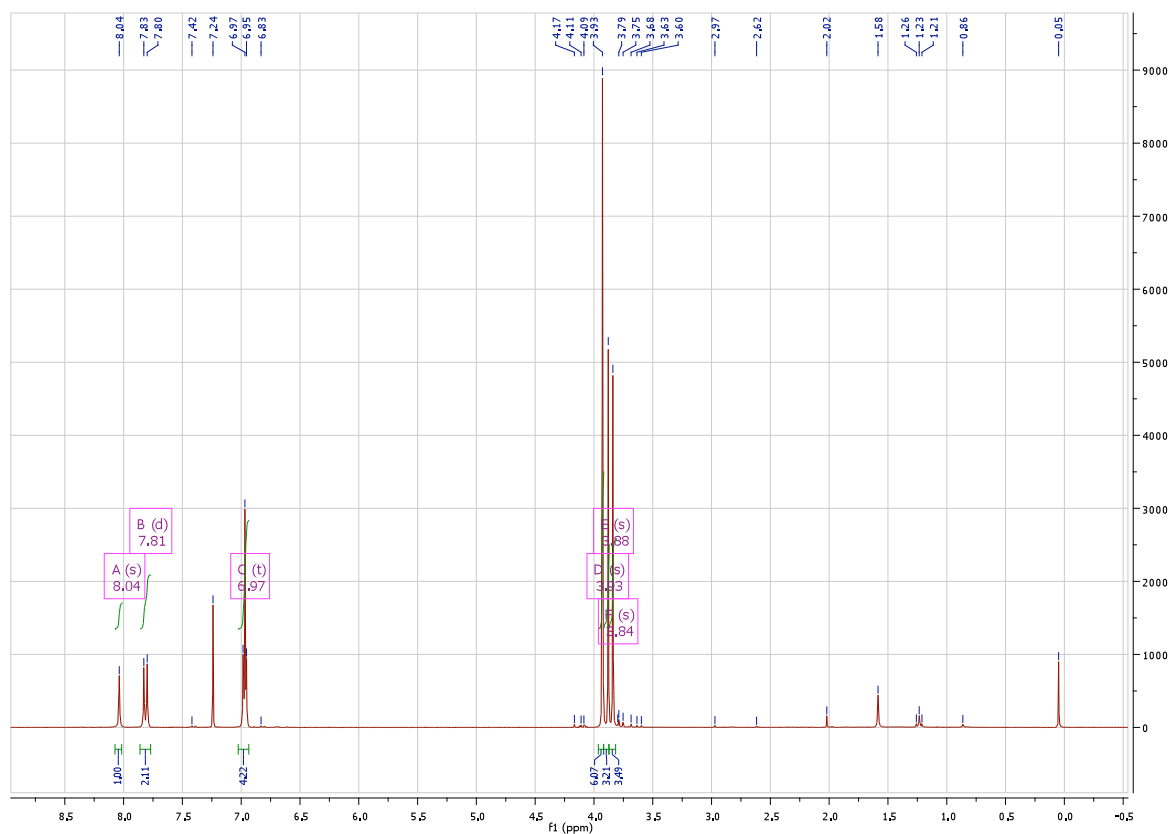
6.12. 1-(4-Methoxy-2-nitrophenyl)-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole



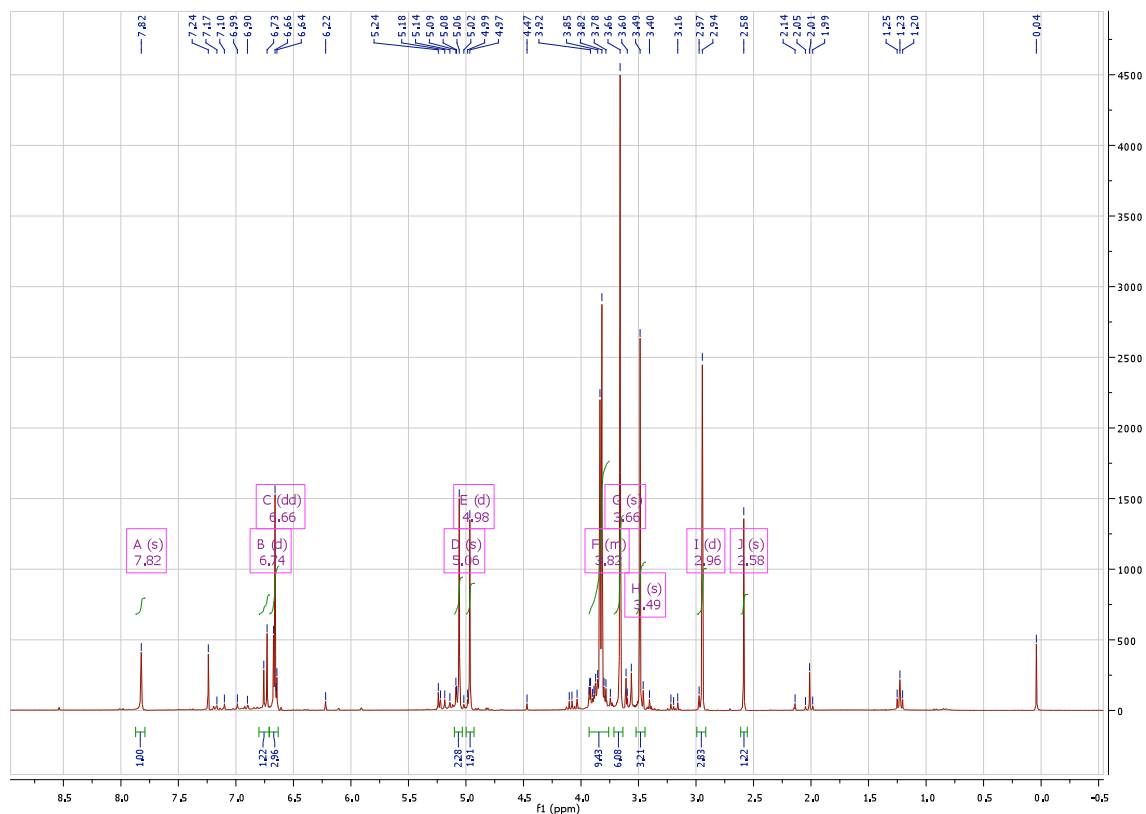
6.13. 5-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole



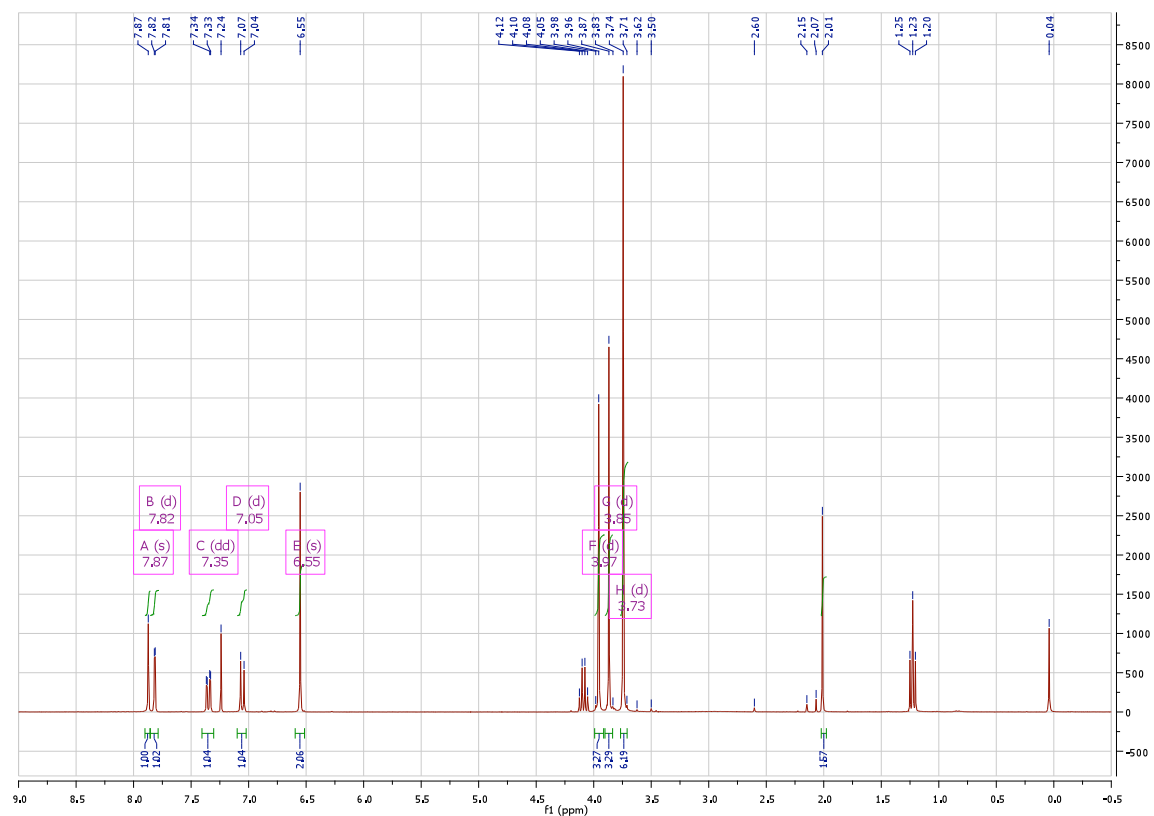
6.14. 4-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole



6.15. 5-(4-methoxy-2,3-bis(MOM)phenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole



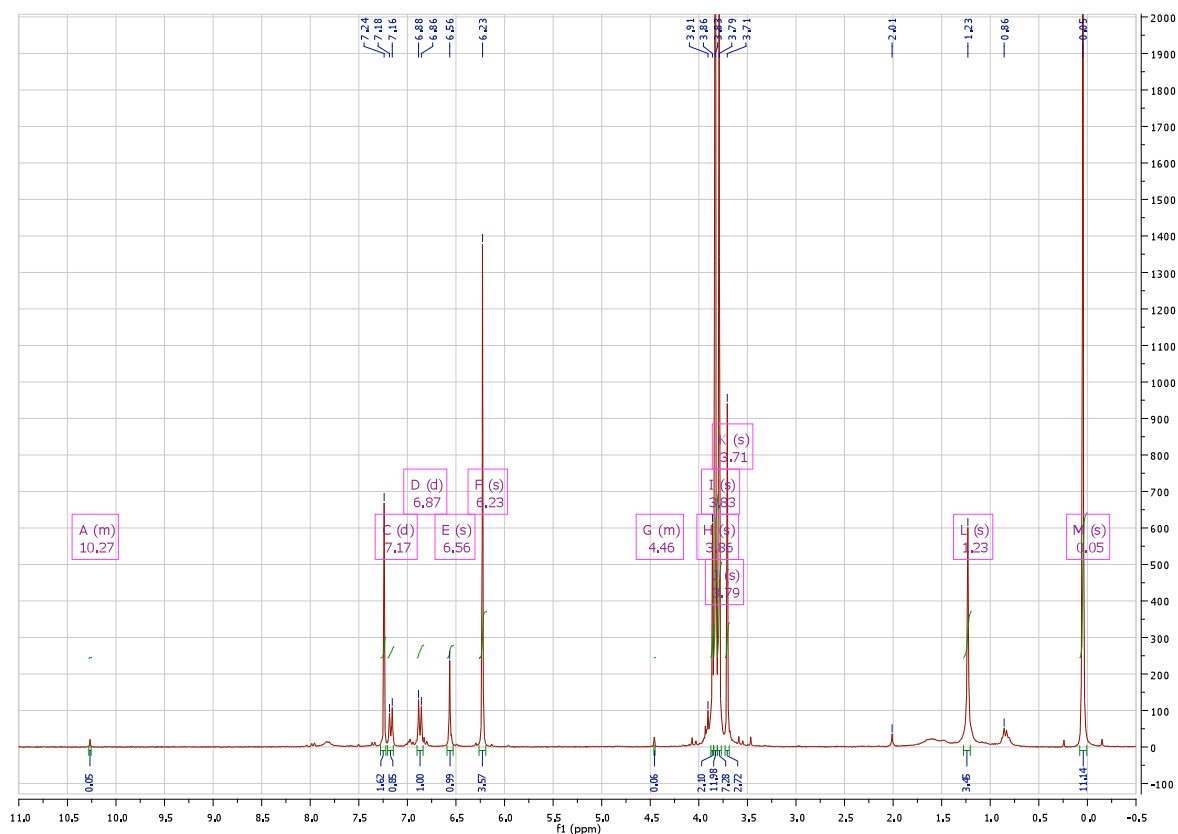
6.16. 5-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole



6.17. Tetra-methyl and tetra-butyl ammonium hydroxide spectra



Appendix Tetra-methyl ammonium hydroxide reaction with trace of 1,5-triazole



Appendix : Tetra-butyl ammonium hydroxide reaction with trace of 1,5-triazole formation